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Award Number: DAMD17-02-P-0588

TITLE: 2002 Congress on In Vitro Biology

PRINCIPAL INVESTIGATOR: John W. Harbell, Ph.D.

CONTRACTING ORGANIZATION: Society for In Vitro Biology
Largo, Maryland 20774

REPORT DATE: June 2002

TYPE OF REPORT: Final Proceedings

PREPARED FOR: U.S. Army Medical Research and Materiel Command
Fort Detrick, Maryland 21702-5012

DISTRIBUTION STATEMENT: Approved for Public Release;
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REPORT DOCUMENTATION PAGE

Form Approved
OMB No. 074-0188

1. AGENCY USE ONLY (Leave blank)	2. REPORT DATE	3. REPORT TYPE AND DATES COVERED
	June 2002	Final Proceedings (6 May 02 - 25 Jun 02)

4. TITLE AND SUBTITLE
2002 Congress on In Vitro Biology

5. FUNDING NUMBERS
DAMD17-02-P-0588

6. AUTHOR(S):
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7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES)
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8. PERFORMING ORGANIZATION REPORT NUMBER

9. SPONSORING / MONITORING AGENCY NAME(S) AND ADDRESS(ES)
U.S. Army Medical Research and Materiel Command
Fort Detrick, Maryland 21702-5012

10. SPONSORING / MONITORING AGENCY REPORT NUMBER

11. SUPPLEMENTARY NOTES
report contains color

12a. DISTRIBUTION / AVAILABILITY STATEMENT
Approved for Public Release; Distribution Unlimited

12b. DISTRIBUTION CODE

13. ABSTRACT ***(Maximum 200 Words)***

none provided

14. SUBJECT TERMS
In Vitro Biology

15. NUMBER OF PAGES
176

16. PRICE CODE

17. SECURITY CLASSIFICATION OF REPORT	18. SECURITY CLASSIFICATION OF THIS PAGE	19. SECURITY CLASSIFICATION OF ABSTRACT	20. LIMITATION OF ABSTRACT
Unclassified	Unclassified	Unclassified	Unlimited

IN VITRO

VOLUME 38 ABSTRACT SPRING 2002

CELLULAR &

DEVELOPMENTAL

BIOLOGY

PROGRAM ISSUE

2002 Congress on In Vitro Biology

June 25 – 29, 2002
Orlando, Florida

Disney's Coronado Springs
Resort

PROGRAM ISSUE

Journal

of the

Society for

In Vitro

Biology

2002 Congress on In Vitro Biology

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About the Issue...

The 2002 Congress on In Vitro Biology focuses on issues pertinent to the Vertebrate, Invertebrate, and Cellular Toxicology Sections and gives participants a unique learning experience on animal cell culture and biotechnology. The 2002 Congress will be held from June 25 – 29, 2002, at *Disney's Coronado Springs* Resort at *Walt Disney World*® Resort.

The Society's Plant Section will meet in conjunction with the International Association for Plant Tissue Culture & Biotechnology (IAPTC&B) for their 10th IAPTC&B Congress – "Plant Biotechnology 2002 and Beyond" on June 23 – 28, 2002 at *Disney's Coronado Springs* Resort in Florida. The IAPTC&B, founded in 1963, with over 2,000 members in more than 65 countries, is the largest, oldest, and most comprehensive international professional organization in the field of plant tissue culture an biotechnology. The 10th IAPTC&B Congress is the first congress to be held at the beginning of the 21st century and the 3rd millennium.

The abstracts for poster presentations from the 10th IAPTC&B Congress are included in this abstract issue. All oral presentations at the 10th IAPTC&B Congress will be included in a separate book of Proceedings that will be forwarded to all Congress participants later this year. For more information on this publication, please contact the Congress Secretariat at sivb@sivb.org.

IN VITRO
ANIMAL
CELLULAR & DEVELOPMENTAL BIOLOGY

Journal of the
Society for
In Vitro Biology

IN VITRO CELLULAR & DEVELOPMENTAL BIOLOGY (ISSN 1071-2690) is published monthly, except bi-

In Vitro Cellular & Development Biology – Plant
Volume 38 2002
ISSN 1054–5476

Publishing, Production, Marketing & Sales Office:
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CAB International
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Oxon OX10 8DE, UK
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Fax: +44 (0)1491 829292
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Toll-free: 1-800 528 4841
Fax: +1 212 686 7993
Email: cabi-nao@cabi.org
CAB International also has regional offices in Malaysia, Trinidad & Tobago and Kenya.
Publishing Director: Philip Edge
Publisher, Plant Sciences: Liz Bennett
Product Manager (Marketing): Penny Orford

SUBSCRIPTION INFORMATION (Annual rates)
In Vitro Cellular & Developmental Biology — Plant is an international journal published bimonthly (one volume, six issues) by CABI *Publishing* (a division of CAB International) on behalf of the Society for In Vitro Biology.
2002, Volume 38
Internet/Print package
$255 USA/Canada/Mexico
$302/£182 Rest of the World
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Internet only
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Japanese agent: Maruzen Company Ltd., PO Box 5050, Tokyo International, 100-31, Japan.
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Back volumes 1991–2001 (as available) can be purchased directly from the Society for In Vitro Biology, 9315 Largo Drive West, Suite 255, Largo, MD 20774, USA or www.sivb.org. Back issues from 2000 onwards can be purchased from CABI *Publishing*.
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This journal is printed on acid-free paper from renewable sources. Typeset and printed by Alden Press, Oxford and Northampton, UK.

Program at a Glance – SIVB Congress on In Vitro Biology

Time	Tuesday, June 25	Time	Wednesday, June 26	Time	Thursday, June 27	Time	Friday, June 28
7:00 am – 8:00 am	Plant Editorial Board Meeting *Cancun* Strategic Long-Range Planning Committee Meeting *Coronado M*	**7:00 am – 8:00 am**	Registration Central – 7:00 am – 6:00 pm CABI/SIVB Meeting *Acapulco* Plant Program Committee Meeting *Baja*	**7:00 am – 8:00 am**	Registration Central – 7:00 am – 7:00 pm Student Affairs Breakfast *Acapulco* Publications Committee Meeting *Baja*	**7:00 am – 8:00 am**	North Registration – 7:00 am – 4:00 pm Development Committee Meeting *Coronado Q* Membership Committee Meeting *Coronado P*
8:00 am – 12:00 pm	Current Board of Directors Meeting *Coronado M*	**8:00 am – 9:00 am**	Basic Culture Techniques (IP) *Exhibit Hall*	**8:00 am – 10:00 am**	Tissue Engineering: How Well We Are Doing (TS) *Durango 1-2*	**8:00 am – 9:00 am**	Preparation for Live-Cell Imaging – Cool Technologies: Workshop (W) *Acapulco*
12:00 pm – 2:00 pm	Incoming Board of Directors Meeting *Coronado M*	**9:00 am – 10:00 am**	Differentiated Epithelium (IP) *Exhibit Hall*	**10:00 am – 10:30 am**	Coffee Break *Exhibit Hall*	**9:00 am – 10:00 am**	Strategies & Technology for Proteomic Analysis of Eucaryotic Cells – Cool Technologies: Workshop (W) *Baja*
		10:00 am – 10:30 am	Coffee Break *Exhibit Hall*				
2:00 pm – 3:00 pm	2002 Program Planning Committee Meeting *Coronado N*	**10:30 am – 12:30 pm**	Is it Good Enough: - An Exploration of Practical Approaches Available for Assessing the Predictive Capacity of In Vitro Tests (TS) *Durango 1-2*	**10:30 am – 12:30 pm**	Microgravity Cell Science (VS) *Durango 1-2*	**10:00 am – 10:30 am**	Coffee Break *Acapulco/Baja/Cancun Foyer*
						10:30 am – 12:30 pm	Vertebrate/Cellular Toxicology Contributed Papers (CP) *Cancun* Proliferation and Differentiation Mechanisms of Insect Stem Cells and Turtle Gonad Cells (CP) *Coronado P*
3:00 pm – 4:00 pm	2003 Program Planning Committee Meeting *Coronado N*	**12:30 pm – 1:00 pm**	Lunch *Exhibit Hall*	**12:30 pm – 1:00 pm**	Lunch *Exhibit Hall*		
4:30 pm – 8:30 pm	**Registration** ***Registration Central***	**1:00 pm – 3:00 pm**	Stem Cells and Organogenesis (JVITS) *Durango 1-2*	**1:00 pm – 2:00 pm**	Invertebrate and Fish Cell Culture: Generation and Application (IP) *Exhibit Hall* Cancer Cells (IP) *Exhibit Hall*	**1:30 pm – 3:30 pm**	In Vitro Approaches to Production of Marine-derived Drugs (JIVS) *Durango 1-2*
4:30 pm – 8:30 pm	Poster Set-up *Exhibit Hall*						
5:00 pm – 6:00 pm	Education Committee Meeting *Coronado M*	**3:30 pm – 5:30 pm**	Plenary Session and Lifetime Achievement Award Presentations (PS) *Durango 1-2*	**5:45 pm – 6:45 pm**	SIVB Business Meeting *Cancun*	**5:00 pm – 8:30 pm**	Closing Banquet *Disney's Animal Kingdom®*
		5:30 pm – 7:00 pm	Plenary and Lifetime Achievement Award Reception *Ventanas Patio West*	**7:00 pm – 9:00 pm**	Biological and Chemical Terrorism: Our Risks and Our Responses (JVTS) *Durango 1-2*	**SPECIAL SATURDAY EVENT**	**Saturday, June 29** Harbor Branch Oceanographic Institute Tour 8:30 am – 4:30 pm
7:00 pm – 8:00 pm	History Society Meeting *Cancun*	**7:30 pm – 9:30 pm**	Plant Business Meeting and Social *Monterey 1-2* Vert/Tox Business Meeting and Social *Acapulco* Invertebrate Business Meeting and Social *Off Property*				

LEGEND

CP – Contributed Paper Session
IP – Interactive Poster Session
JIVS – Joint Invert/Vert Symposium
JVITS – Joint Vert/Invert/Tox Symposium
JVTS – Joint Vert/Tox Symposium
PS – Plenary Session
TS – Toxicology Symposium
VS – Vertebrate Symposium
W – Joint Vert/Invert/Tox Workshop

Schedule of Functions

TIME	TYPE OF FUNCTION	ROOM
TUESDAY, JUNE 25		
7:00 am – 8:00 am	In Vitro – Plant Editorial Board Meeting	Cancun
7:00 am – 8:00 am	Strategic Long-Range Planning Committee Meeting	Coronado M
8:00 am – 12:00 pm	SIVB Current Board of Directors Meeting	Coronado M
12:00 pm – 2:00 pm	SIVB Incoming Board of Directors Meeting	Coronado M
2:00 pm – 3:00 pm	2002 Program Planning Committee Meeting	Coronado N
3:00 pm – 4:00 pm	2003 Program Planning Committee Meeting	Coronado N
4:30 pm – 8:30 pm	**Registration**	Registration Central
4:30 pm – 8:30 pm	Poster Set-up	Exhibit Hall
5:00 pm – 6:00 pm	Education Committee Meeting	Coronado M
7:00 pm – 8:00 pm	History Society Meeting	Cancun
WEDNESDAY, JUNE 26		
7:00 am – 6:00 pm	Registration	Registration Central
7:00 am – 8:00 am	SIVB/CABI Business Meeting	Acapulco
7:00 am – 8:00 am	Plant Program Committee Meeting	Baja
8:00 am – 10:00 pm	**Exhibits and Posters**	Registration Central
10:00 am – 10:30 am	Coffee Break	Exhibit Hall
12:30 pm – 1:00 pm	Lunch	Exhibit Hall
5:30 pm – 7:00 pm	Plenary and Lifetime Achievement Awards Reception	Ventanas Patio West
7:30 pm – 9:30 pm	Plant Business Meeting and Social	Monterey 1-2
7:30 pm – 9:30 pm	Vertebrate/Toxicology Business Meeting and Social	Acapulco
7:30 pm – 9:30 pm	Invertebrate Business Meeting and Social	Off Property
THURSDAY, JUNE 27		
7:00 am – 7:00 pm	Registration	Registration Central
7:00 am – 8:00 am	Student Affairs Breakfast	Acapulco
7:00 am – 8:00 am	Publications Committee Meeting	Baja
8:00 am – 2:00 pm	**Exhibits and Posters**	Exhibit Hall
10:00 am – 10:30 am	Coffee Break	Exhibit Hall
12:30 pm – 1:00 pm	Lunch	Exhibit Hall
2:00 pm – 3:00 pm	Poster Breakdown and Removal	Exhibit Hall
5:45 pm – 6:45 pm	SIVB Business Meeting	Cancun
FRIDAY, JUNE 28		
7:00 am – 4:00 pm	Registration	North Registration
7:00 am – 8:00 am	Development Committee Meeting	Coronado Q
7:00 am – 8:00 am	Membership Committee Meeting	Coronado P
10:00 am – 10:30 am	Coffee Break	Acapulco/Baja/Cancun Foyer
5:00 pm – 8:30 pm	Closing Banquet	*Disney's* Animal Kingdom®
SATURDAY, JUNE 29		
8:30 am – 4:30 pm	Harbor Branch Oceanographic Institute Tour	Harbor Branch Oceanographic Institute

Note: Additions and changes to functions will be posted on a bulletin board located in the registration area. Please check the bulletin board daily.

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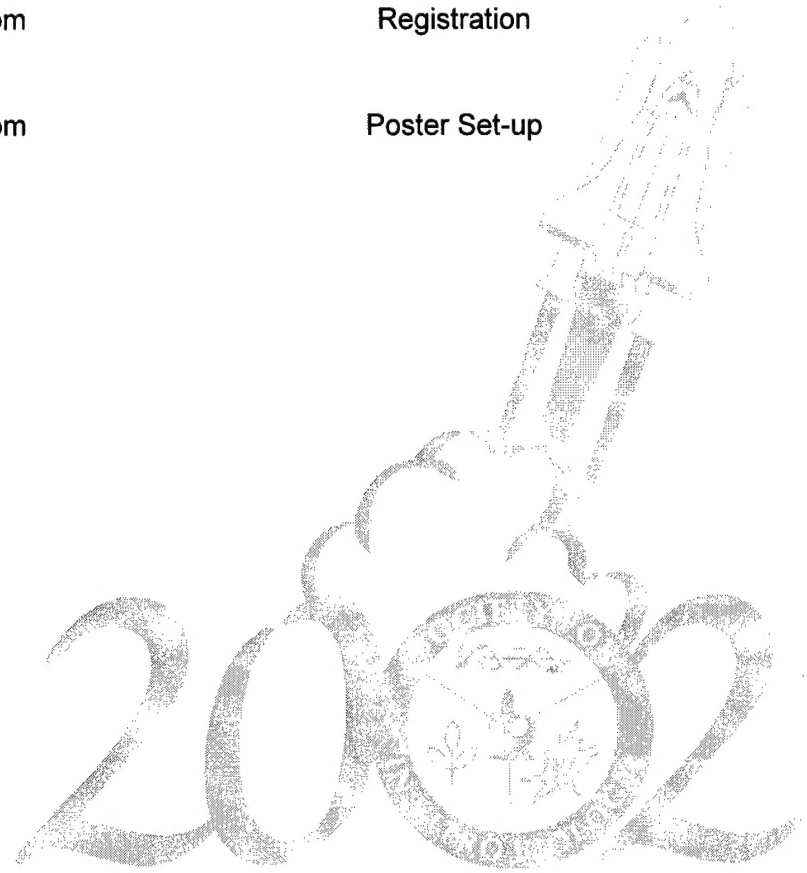
Marietta Ellis, Society for In Vitro Biology

Tuesday, June 25

8:00 am – 12:00 pm	SIVB CURRENT BOARD OF DIRECTORS MEETING	Coronado M
12:00 pm – 2:00 pm	SIVB INCOMING BOARD OF DIRECTORS MEETING	Coronado M
4:30 pm– 8:30 pm	Registration	Registration Central
4:30 pm– 8:30 pm	Poster Set-up	Exhibit Hall



Wednesday, June 26

7:00 am – 6:00 pm	Registration	Registration Central
8:00 am – 10:00 pm	Exhibits and Posters	Exhibit Hall

BASIC CULTURE TECHNIQUES

Moderator: Lia H. Campbell, Organ Recovery Systems

8:00 am – 9:00 am Interactive Vertebrate/Toxicology Poster Session (See abstract pages 10-A to 11-A) Exhibit Hall

Interactive Poster Sessions will begin with each poster presenter giving a short description of their work (5-minutes maximum). After a selected number of introductions have been completed (as determined by the moderator), the floor will be opened for discussion. We hope this will be a positive experience for presenters, poster viewers, and exhibitors alike.

PART A: CELL IDENTIFICATION AND PRESERVATION

VT-1000 Authenticity of Animal Cell Culture by PCR and DNA Sequencing Analysis
Merry Yinmei Liu, *Center for Disease Control and Prevention, S.-C. Lin, A. Vafia, and F. Candal*

VT-1001 Identification System for Cross-contamination in Cultured Cell Lines by Combined Methods with STR-PCR and Molecular Cytogenetics in JCRB
Hideyuki Tanabe, *National Institute of Health Sciences, R. Iizuka, M. Hojo, M. Kurematsu, Y. Takada, T. Masui, and H. Mizusawa*

VT-1002 Extracellular Matrix Enhances the Survival of Cryopreserved Adherent Cells
Lia H. Campbell, *Organ Recovery Systems, K. Sarver, M. J. Taylor, and K. G. M. Brockbank*

PART B: DIFFERENTIATION

VT-1003 Strategies for Improving the Cell Culture Medium Performance
Vijai K. Pasupuleti, *Sai International, A. Moore, and N. Savich*

VT-1004 Multiple Defined Human Cell-biomatrix Coculture Models for Tissue Growth and Differentiation Studies
Raj K. Singh, *University of Alabama at Birmingham, P. Collingsworth, V. Alapati, X. Chen, and G. P. Siegal*

VT-1005 Role of HGF/SF in Somite Myogenesis
Masataka Shiozuka, *Waseda University, S. Yokoyama, and I. Kimura*

VT-1006 Effects of Murine Bone Marrow Endothelial Cell Conditioned Medium on the Growth of Yolk Sac Hematopoietic Progenitors and Angioblasts
Qiru Wang, *Central South University, X. D. Na, and Q. Y. Xie*

VT-1007 Bone Marrow Endothelial-cell-derived Factors Inhibiting the Growth of Bone-marrow-derived Fibroblast Colony-forming Cells (CFU-F)
Baohe Wang, *Hunan Normal University, and Q. R. Wang*

DIFFERENTIATED EPITHELIUM

Moderator: John W. Harbell, Institute for In Vitro Sciences, Inc.

9:00 am – 10:00 am Interactive Vertebrate/Toxicology Poster Session Exhibit Hall
(See abstract pages 11-A to 13-A)

Interactive Poster Sessions will begin with each poster presenter giving a short description of their work (5-minutes maximum). After a selected number of introductions have been completed (as determined by the moderator), the floor will be opened for discussion. We hope this will be a positive experience for presenters, poster viewers, and exhibitors alike.

VT-1008 Dynamics of Epithelium Integrity by 3D Rendering of EGF/erb-B1 Complexes
Bertrand A. Kaeffer, *Institut National Recherche, A. Trubuil, C. Kervrann, L. Pardini, and C. Cherbut*

VT-1009 Phosphorylation of S146 and S153 at p21 by Protein Kinase C in Keratinocytes
Mariko Kashiwagi, *Showa University, and T. Kuroki*

VT-1010 RAD, a Small GTP Protein, is Identified as a Transdifferentiation Factor that Inhibits Mucous Cell Differentiation but Stimulates Squamous Cell Differentiation in Airway Epithelium
Reen Wu, *University of California at Davis, X. G. Shao, and M. J. Chang*

VT-1011 An In Vitro Model for the Rapid Screening of Potential Components and Formulations for Nasal Drug Delivery
Elizabeth Scotto-Lavino, *State University of New York at Stony Brook, J. M. Easow, S. R. Simon, and E. J. Roemer*

VT-1012 Evaluation of the Penetration and Cytotoxic Effects of Drug Formulations on an In Vitro Nasal Mucosal Model
Jeena Marian Easow, *State University of New York at Stony Brook, E. Scotto-Lavino, S. R. Simon, and E. J. Roemer*

VT-1013 Early Markers of Cell Injury Following In Vitro Sulfur Mustard Exposure in Human Epidermal Keratinocytes
William J. Smith, *US Army Medical Research Institute of Chemical Defense, O. E. Clark III, and E. W. Nealley*

VT-1014 Preliminary Evaluation of a Cytotoxicity Model for Predicting Acute Oral Toxicity Test Starting Doses
John W. Harbell, *Institute for In Vitro Sciences, Inc., G. Mun, R. Ruppalt, and R. D. Curren*

10:00 am – 10:30 am Coffee Break Exhibit Hall

IS IT GOOD ENOUGH? – AN EXPLORATION OF PRACTICAL APPROACHES AVAILABLE FOR ASSESSING THE PREDICTIVE CAPACITY OF IN VITRO TESTS

Convener: **Leon Bruner,** Gillette Medical Evaluation Laboratories

10:30 am – 12:30 pm Toxicology Symposium Durango 1-2
(See abstract page 4-A)

The goal of many in vitro studies is the quantitative prediction of a biological response in the tissues or whole organisms. Examples of such applications include studies of pharmaceutical action, herbicidal

action, anti-neoplastic activity, endocrine disruption, and organ toxicity. In almost all cases, the in vitro test is compared to an in vivo reference test in a validation study. Approaches to the design and conduct of validation studies have become clearer because of the considerable work done to develop and validate toxicity tests in recent years. However, an area that has required further research is how best to measure test method performance in validation studies and how to set criteria that should be used to judge the adequacy of this performance. Traditional methods of dividing data into positive and negative categories and then calculating performance statistics such as sensitivity and specificity can provide misleading information. This symposium will review the results of recent research designed to examine the information obtained from methods most commonly used to assess in vitro test performance. The presentations will outline the advantages and disadvantages of these methods. The presentations will also share recently discovered misconceptions related to the interpretation of results from in vivo/in vitro comparisons. The symposium will focus on practical examples that will be particularly useful for scientists involved in the development and validation of in vitro tests designed to predict biological responses.

10:30 Introduction (L. Bruner)
10:45 T-1 Practical Comparison of Methods Used to Measure the Predictive Capacity of In Vitro Tests
Leon Bruner, *Gillette Medical Evaluation Laboratories*
11:15 T-2 Evaluating Test Methods by Their Estimated Performance
Rodger D. Curren, *Institute for In Vitro Sciences, Inc.*
11:45 T-3 Is My Assay Good Enough? Experience from a Real-world Validation
Jacqueline Southee, *Gillette Medical Evaluation Laboratories*
12:15 Discussion

12:30 – 1:00 Lunch Exhibit Hall

STEM CELLS AND ORGANOGENESIS

Conveners: Raziel S. Hakim, Howard University
Nam-ho Huh, Okayama University, Japan

1:00 pm – 3:00 pm Joint Vertebrate/Invertebrate/Toxicology Symposium Durango 1-2
(See abstract page 2-A)

With the rapid accumulation of information on the capabilities of stem cells, many questions remain about the diversity of cell types that can form from adult-derived stem cell populations and the processes that control the diverse cell fates and differentiation steps in adult systems. The speakers will address the question of stem cell capabilities, by describing the developmental capacities of bone marrow derived mesenchymal stem cells, and how these capabilities can be triggered by their environments in vitro. A second major issue concerns how stem cells form organs. Speakers will also describe vascular stem cells and early processes involved in angiogenesis.

1:00 Introduction (R. Hakim)
1:15 J-1 Studying Blood Vessel Development Using the Zebrafish
Brant Weinstein, *National Institutes of Health*
1:40 J-2 Differentiation of Rat Bone Marrow Cells into Hepatocytes in Culture
Masahiro Miyazaki, *Okayama University*
2:05 J-3 Molecular Target for the Regulation of Angiogenesis
Yasufumi Sato, *Tohoku University*
2:30 J-4 Human Mesenchymal Stem Cells Differentiate to Multiple Lineages
Mark Pittenger, *Osiris Therapeutics, Inc.*

PLENARY SESSION AND LIFETIME ACHIEVEMENT AWARD PRESENTATIONS

3:30 – 5:30 Plenary Session Durango 1-2

Conveners: Cynthia L. Goodman, 2002 Program Committee Co-Chair
Lia H. Campbell, 2002 Program Committee Co-Chair

3:30 Welcome and Announcements

LIFETIME ACHIEVEMENT AWARD PRESENTATIONS

3:35 Introduction Mary Ann Lila Smith, University of Illinois and President, Society for In Vitro Biology

Lifetime Achievement Award Recipients:
Gordon A. Sato, Department of Fisheries, Masawa, Eritrea
Sadar S. Sohi, Natural Resources Canada, Canadian Forest Service, Canada

PLENARY SESSION

Commercializing Our Biotechnologies
(See abstract page 1-A)

Conveners: Shirley Pomponi, Harbor Branch Oceanographic Institute
Lia H. Campbell, Organ Recovery Systems

4:00 pm Opening Remarks: Shirley Pomponi, Harbor Branch Oceanographic Institute

PS-1 Plenary Speaker: **Sheldon Schuster,** Director of the Biology Program, Interdisciplinary Center for Biotechnology Research, University of Florida in Gainesville

DISTINGUISHED PLENARY SESSION

The Manzanar Project: Contributions of In Vitro Biology, Tissue Engineering, Proteomics, and Beyond
(See abstract page 1-A)

Conveners: Wallace L. McKeehan, Texas A&M University Health Science Center
Sandra L. Schneider, Research and Clinical Lab Systems

4:35 pm Opening Remarks: Wallace L. McKeehan, Texas A&M University Health Science Center

PS-2 Plenary Speaker: **Gordon Sato,** Department of Fisheries, Masawa, Eritrea

PLENARY AND LIFETIME ACHIEVEMENT AWARDS RECEPTION

5:30 pm – 7:00 pm Ventanas Patio West

Thursday, June 27

7:00 am – 7:00 pm | Registration | Registration Central

8:00 am – 2:00 pm | Exhibits and Posters | Exhibit Hall

TISSUE ENGINEERING: HOW WELL WE ARE DOING

Conveners: Gordana Vunjak-Novakovic, Massachusetts Institute of Technology
Jonathan Garlick, State University of New York, Stony Brook

8:00 am – 10:00 pm | Toxicology Symposia (See abstract pages 4-A to 5-A) | Durango 1-2

Tissue engineering can potentially address tissue and organ failure by allowing implantation of an engineered construct that is functional from the start and has the capacity to integrate (structurally and functionally) with the host tissues. In vitro, engineered tissues can serve as physiologically relevant models to identify and quantitate biological mechanisms inherent during tissue development, and to distinguish between the specific effects of cell-derived, biochemical and physical signals. The clinical and scientific relevance of tissue engineering critically depends on our ability to direct cells to form specialized tissues. This session will attempt to address the scientific, engineering, and clinical aspects of tissue engineering, and to evaluate how well are we doing with the in vitro cultivation of functional tissue equivalents.

8:00 Introduction (G. Vunjak-Novakovic and J. Garlick)
8:15 T-4 Lifespan Extension in Vascular Tissue Engineering
Laura E. Niklason, *Duke University*
8:45 T-5 Engineering Human Tissue Models to Study Basement Membrane Biology
J. Garlick, *State University of New York – Stony Brook*
9:15 T-6 Quantitative In Vitro Studies of Tissue Development
G. Vunjak-Novakovic, *Massachusetts Institute of Technology*
9:45 Discussion

10:00 am – 10:30 am | Coffee Break | Exhibit Hall

MICROGRAVITY CELL SCIENCE

Conveners: J. Milburn Jessup, Georgetown University
Neal R. Pellis, NASA-Johnson Space Center

10:30 am – 12:30 pm | Vertebrate Symposium (See abstract page 6-A) | Durango 1-2

Cells respond to the low gravity of space by unique gene expressions and metabolic adaptations. Modeling microgravity is sought through the use of analog cell culture systems. This session will present investigations that demonstrate: 1) the advantages in tissue morphogenesis in microgravity analog culture,

2) models of human disease, and 3) the underlying mechanisms of the cellular response to changes in gravity.

10:30 Introduction (J. M. Jessup and N. R. Pellis)

10:45 V-1 Microgravity Analog Culture as a Model for Lymphocyte Performance in Space
***Neal R. Pellis**, NASA-Johnson Space Center*

11:10 V-2 Use of NASA-designed Bioreactor for Organotypic Liver Cultures of Human Colorectal Carcinoma Metastases
***J. Milburn Jessup**, Georgetown University Medical Center*

11:35 V-3 Effects of Shear and Gravity on Renal Cells
***Timothy G. Hammond**, Tulane University Health Sciences Center*

12:00 V-4 Blood Cells and Space Biology
***Arthur J. Sytkowski**, Beth-Israel Deaconess Medical Center*

12:30 pm – 1:00 pm Lunch Exhibit Hall

INVERTEBRATE AND FISH CELL CULTURE: GENERATION AND APPLICATION OF CELL LINES

1:00 pm – 2:00 pm Joint Interactive Invertebrate/ Vertebrate/Toxicology Poster Session (See abstract pages 16-A to 17-A) Exhibit Hall

Moderator: Guido Caputo, Natural Resources Canada, Canadian Forest Service

Interactive Poster Sessions will begin with each poster presenter giving a short description of their work (5-minutes maximum). After a selected number of introductions have been completed (as determined by the moderator), the floor will be opened for discussion. We hope this will be a positive experience for presenters, poster viewers, and exhibitors alike.

JI-1000 Novel Insect Primary Culture Method by Using Newly Developed Media and Extra Cellular Matrix
***Shigeo Imanishi**, National Institute of Agrobiological Sciences, G. Akiduki, and M. Haga*

JI-1001 Development and Testing of Insect Cell Lines for Neuronal Characteristics
***Amy Wang**, GlaxoSmithKline, C. L. Goodman, USDA-ARS-BCIRL, A. H. McIntosh, H. Nabli, and J. Wittmeyer*

JI-1002 Synthetic Activity of Cultured Corpora Cardiaca/Corpora Allata Complexes from the Two-spotted Stinkbug
***Cynthia L. Goodman**, USDA-ARS-BCIRL, R. M. Wagner, H. Nabli, D. Davis, S. Crimmins, and T. Okuda*

JV-1003 Effect of Poly IC on Salmonid Cell Lines
***Stephanie Johanna De Witte-Orr**, University of Waterloo, L. E. J. Lee, and N. C. Bols*

JT-1004 Toxicity of Alkylated Naphthalenes to a Rainbow Trout Gill Cell Line
***Vivian Rashida Dayeh**, University of Waterloo, G. Jeremic, S. Lee, K. Schirmer, P. V. Hodson, and N. C. Bols*

CANCER CELLS

Moderator: June A. Bradlaw, International Foundation for Ethical Research

1:00 pm – 2:00 pm Interactive Vertebrate/Toxicology Poster Session Exhibit Hall
(See abstract pages 13-A to 15-A)

Interactive Poster Sessions will begin with each poster presenter giving a short description of their work (5-minutes maximum). After a selected number of introductions have been completed (as determined by the moderator), the floor will be opened for discussion. We hope this will be a positive experience for presenters, poster viewers, and exhibitors alike.

PART A: REGULATION OF TUMOR CELLS

VT-1015 Potential Use of J774A.1 Macrophage Cells to Biomonitor Estrogenic Activity in Non-estrogen Dependent Tissue
Quentin H. Felty, *University of Alabama at Birmingham, and D. Roy*

VT-1016 Regulation of Growth of Human Embryonic Kidney Cells Through a Novel Alu Sequence Repeat Containing Gene
Kamaleshwar P. Singh, *University of Alabama at Birmingham, and D. Roy*

VT-1017 Analysis of Cullin-5/VACM-1 mRNA Expression in Breast Epithelial Cells, Breast Cancer Cell Lines, and Both Normal and Tumor Tissues
Michael J. Fay, *Midwestern University, G. A. Karathanasis, C. J. Mandernach, J. R. Leong, A. Hicks, K. Pherson, and A. Hussain*

VT-1018 Modulation of Fas Expression and Sensitivity to Fas-induced Apoptosis in Human Cancerous Pancreatic Ductal Cells Maintained in Culture and Xenografted in Nude Mice
Soroosh Radfar, *Université Paul Sabatier, Christian Davrinche, and E. Hollande*

PART B: EVALUATION OF ANTI-CANCER DRUGS

VT-1019 The Kinetics of Matrix Metalloproteinase Inhibition by Chemically Modified Tetracyclines
Lisa C. Chen, *State University of New York at Stony Brook, W. J. Bellucci, S. R. Simon, and E. J. Roemer*

VT-1020 The Effects of Chemically Modified Tetracyclines (CMTs) on MonoMac-6 Cell Secretion of Matrix Metalloproteinase-9 (MMP-9) and Tissue Inhibitor of MMP (TIMP)
Sophia Khan, *State University of New York at Stony Brook, S. R. Simon, and E. J. Roemer*

VT-1021 Tumorigenicity and Toxicity Assessment of Thalidomide Alone and in Combination with Cisplatin in Mice and Cultured Murine Cells
Shyamal K. Majumdar, *Lafayette College, and J. M. Ruddy*

2:00 pm – 3:00 pm Poster Breakdown and Removal Exhibit Hall

5:45 pm – 6:45 pm **SIVB Business Meeting** Cancun
(All are urged to attend)

BIOLOGICAL AND CHEMICAL TERRORISM: OUR RISKS AND OUR RESPONSES

Convener: William J. Smith, US Army Medical Research Institute of Chemical Defense

7:00 pm – 9:00 pm Joint Vertebrate/Toxicology Symposium Durango 1-2

The threat of chemical and biological attacks has been with society from the earliest times. The chemical attacks on the Japanese subway system and the anthrax attacks in the US have brought home the message that such threats are not historical anecdotes, but painful reality. The scientific community has both personal and professional obligations in the face of these threats. Our family, friends, and neighbors call us for guidance as to what they should do. Our communities look to us for answers. We need to know what are the risks we face and what are the responses we have to provide in the event of a terrorist attack. The speakers are actively involved in the chemical and biological defense arena and will be presenting specific information on these threats, but will also leave significant time for audience participation and questions.

7:00		Introduction (W. J. Smith)
7:05	J-5	The Combined Human/Agricultural Bioterrorism Threat ***Richard Meyers,*** *USDA*
7:45	J-6	Chemical Warfare and Environmental Chemical Threats ***William J. Smith,*** *US Army Medical Research Institute of Chemical Defense*
8:05		Discussion

Friday, June 28

7:00 am – 4:00 pm Registration North Registration

PREPARATION FOR LIVE-CELL IMAGING
COOL TECHNOLOGIES: WORKSHOP

Convener: Lia Campbell, Organ Recovery Systems

8:00 am – 9:00 am Joint Vertebrate/Invertebrate/Toxicology Workshop Acapulco

We are in the early stages of a revolution in microscopy. Advances in microscopes and microscope-related technology are being applied to answer questions relative to live cell behavior thus providing answers not possible from fixed cells. A workshop entitled Preparation for Live-Cell Imaging will include brief discussions relative to the integration of various modes and/or combinations of modes of microscopy such as, brightfield, darkfield, phase, DIC, VAREL, Hoffman, Fluorescence, Confocal and Multiphoton as applied to time lapse imaging of live organisms. The workshop will also include an overview of the physical characteristics and limitations of various micro-environmental control techniques as well as introduce new technologies destined to change microscopy.

8:00 Introduction (L. Campbell)
8:15 Preparation for Live-Cell Imaging
Dan Focht, *Bioptechs*

STRATEGIES AND TECHNOLOGY FOR PROTEOMIC ANALYSIS OF EUCARYOTIC CELLS
COOL TECHNOLOGIES: WORKSHOP

Convener: Lia Campbell, Organ Recovery Systems

9:00 am – 10:00 am Joint Vertebrate/Invertebrate/Toxicology Workshop Baja

Proteomics encompasses the study of multiple proteins that comprise particular biological subsystems, such as various types of cells or sub-cellular complexes. While two-dimensional gel electrophoresis is the traditional analytical tool for evaluating a complex mixture of proteins, other methods being developed offer increased speed, sensitivity, and reproducibility. In particular, instruments coupling liquid chromatography (LC) with tandem mass spectrometry (MS/MS) enable the rapid detection of many proteins without a requirement for prior purification. This presentation will include a discussion of sample preparation methods for eucaryotic cells and cell sub-fractions, an introduction to proteomic analysis using mass spectrometry, and examples of the use of LC-MS/MS for proteomic analysis of purified human T-cell and liver cell subtypes. The system is particularly advantageous for analysis of proteins associated with cell membranes. The technology is most useful for cells from organisms which have more complete genomic sequence information, but additional tools for de novo peptide sequence determination are making it possible to analyze nearly any sample.

9:00 Introduction (L. Campbell)
9:15 Strategies and Technology for Proteomic Analysis of Eucaryotic Cells
Colette Rudd, *Thermo Finnigan*

10:00 am – 10:30 am Coffee Break Acapulco/Baja/Cancun Foyer

VERTEBRATE/CELLULAR TOXICOLOGY CONTRIBUTED PAPERS

Moderator: Kim O'Connor, Tulane University

10:30 am – 12:30 pm Joint Vertebrate/Toxicology Contributed Paper Session Cancun
(See abstract pages 7-A to 8-A)

PART A: MICROGRAVITY BIOREACTOR CULTURES

10:30 CP-1 Mechanobiologic Research Bioreactor
Andrea Guidi, *Universita degli Studi di Napoli*

10:45 CP-2 Modeled Microgravity Culture of Pancreatic Islets for Transplantation Improves Morphology and Function
Lynne P. Rutzky, *University of Texas Health Science Center Medical School, M. Kloc, S. Bilinski, T. Phan, H. Zhang, S. Katz, and S. Stepkowski*

11:00 CP-3 Calcium Signaling Effects of Modeled Microgravity in Human Lymphocytes: Cell Signaling in Lymphocyte Locomotion
Alamelu Sundaresan, *Johnson Space Center, D. Risin, and N. R. Pellis*

PART B: CANCER CELLS

11:15 CP-4 Spheroid Self-assembly of Well and Poorly Differentiated Prostate Cancer Cells
Kim O'Connor, *Tulane University, R. M. Enmon, H. Song, D. J. Lacks, and D. K. Schwartz*

11:30 CP-5 Premature Senescence or Cell Death: Cell Cycle Checkpoints Determine Cellular Response of Normal Human Fibroblasts to Oxidants
Qin M. Chen, *University of Arizona, T. K. Dilley, V. C. Tu, and S. Purdom*

11:45 CP-6 Impairment of Malignant Tumor Phenotypes by Electrolyzed Reduced Water
Saneteka Shirahata, *Japanese Association of Animal Cell Technology, T. Hara, T. Komatsu, T. Hamasaki, H. Nogami, K. Teruya, S. Morisawa, K. Otsubo, and Y. Katakura*

12:00 CP-7 Effect of Antitumor Activity of *Sargassum siliquosum* on Breast Cancer Cell Line T47D
Marselina Irasonia Tan, *Bandung Institute of Technology, Y. Sandraling, A. Siddiq, A. Barlian, and S. Haga*

12:15 Discussion

PROLIFERATION AND DIFFERENTIATION MECHANISMS OF INSECT STEM CELLS AND TURTLE GONAD CELLS

(See abstract pages 8-A to 9-A)

Moderator: Marcia Joan Loeb, U. S. Department of Agriculture

10:30 am – 11:30 pm Invertebrate Contributed Paper Session Coronado P

10:30 CP-8 Manipulation of Stem Cells from Midguts of *Heliothis virescens* (Lepidoptera) Larvae
Marcia Joan Loeb, *U. S. Department of Agriculture, E. A. Clark, and H. Do*

10:50 CP-9 Effects of a Fat Body Extract on Larval Midgut Cells and Growth in Several Lepidoptera
Kim Elsen, *Free University of Brussels, M. Loeb, and G. Smagghe*

11:10 CP-10 The Synthesis of Heat Shock Proteins During Gonadal Sex Differentiation in Male and Female Green Turtle Cell Cultures
Anggraini Barlian, Institute Technology Bandung, S. Sudarwati, L. A. Sutasurya, and H. Hayashi

IN VITRO APPROACHES TO PRODUCTION OF MARINE-DERIVED DRUGS

Convener: Shirley Pomponi, Harbor Branch Oceanographic Institution

1:30 pm – 3:30 pm Joint Invertebrate/Vertebrate Symposium Durango 1-2

(See abstract page 3-A)

Marine invertebrates are the source of many bioactive compounds with therapeutic potential. A critical issue in drug development for marine natural products is ensuring an adequate supply of compounds for clinical use. This workshop addresses different strategies for developing marine invertebrate cell lines, explants, and three-dimensional aggregates for in vitro production of bioactive compounds.

1:30 Introduction (S. Pomponi)

1:45 J-7 Sustainable Production of Bioactive Compounds from Sponges: Primmorphs as Bioreactors
Werner E. G. Müller, *Institut für Physiologische Chemie*

2:10 J-8 Sponge Cell Cultures: Progress in Medium Design
Salvatore De Rosa, *ICMIB-CNR, Italy*

2:35 J-9 In Vitro Cell Culture from Marine Sponges, a Promising System to Produce Bioactive Compounds
Evelyn Richelle-Maurer, *Université Libre de Bruxelles, Belgium*

3:00 J-10 In Vitro Gene Expression in Marine Sponge Cells Stimulated by Phytohemagglutinin
Robin Willoughby, *Harbor Branch Oceanographic Institution*

5:00 pm – 8:30 pm **Closing Banquet** *Disney's Animal Kingdom®*
Seating is limited. Admittance to Banquet by Advance Ticket Holders only.

INVERTEBRATE SILENT ABSTRACT

I-1000 Maintenance of Midgut Epithelial Cells from *Dentroctonus valens* larvae (Coleoptera:Scolytidae) In Vitro
Laura Sanchez, *Escuela Nacional de Ciencias Biológicas-IPN, J. L. Andrade, R. Cisneros, and G. Zúñiga*

VERTEBRATE SILENT ABSTRACTS

V-1000 Multifaceted Phosphofructokinase and the Apparent Plieotypic Effect in Metabolic and Cell Cycle Control
Marco Rabinovitz, *Biochemically Based Drug Design & Synthesis*

V-1001 Distribution of Carbonic Anhydrase IV (CA IV) in Golgi Compartments of Polarized Human Pancreatic Duct Cells Expressing Wild Type or Mutated (DF508) CFTR (Cystic Fibrosis Transmembrane Conductance Regulator)
C. Salvador-Cartier, *Universite Paul Sabatier, M. Fanjul, and Etienne Hollande*

V-1002 Morphological Survival of Cryopreserved Bovine Oocytes at Different Culture Periods After Thawing
Laura Simonetti, *Universidad Nacional de Lomas de Zamora, and M. R. Blanco*

V-1003 Morphology and In Vitro Fertilization of In Vitro Matured Bovine Oocytes After Cryopreservation Using BSA or FBS in Cryoprotective Solutions
Laura Simonetti, *Universidad Nacional de Lomas de Zamora, and M. R. Blanco*

V-1004 Morphological Survival of Immature and In Vitro Matured Bovine Oocytes After Cryopreservation in Solutions Containing Different Protein Concentrations
María del Rosario Blanco, *Universidad Nacional de Lomas de Zamora, and L. Simonetti*

Notes

PS-1

Commercializing Our Biotechnologies. SHELDON SCHUSTER. University of Florida, Interdisciplinary Center for Biotechnology Research, PO Box 110580, South Newell Drive, Gainesville, FL 32611. Email: schuster@biotech.ufl.edu

The opportunities to bring the benefits of Biotechnology to the marketplace so they will benefit society are increasing constantly. We can work in academic, corporate or government laboratories and still maintain the ability to cure diseases, produce new, safer and more nutritious foods, and help clean and protect the environment. However, proceeding from concept to proof of principle to a real product is a long, difficult and costly process. Therefore, because of the massive public investment in the basic research underlying these technologies, as well as the financial success achieved by a variety of institutions, pressure is steadily increasing to transfer technology from the public to the private sector. Since this is a relatively new endeavour, it is fraught with misunderstanding, unrealistic expectations, and unnecessary difficulties. Many of these problems can be avoided if we understand the differences between the various cultures. In addition, an understanding of the unique and important contributions of scientists and business people is essential if success is possible. We will discuss these issues, and show examples of how this process has succeeded, as well as those instances where it resulted in a less positive outcome. Guidelines for proceeding from discovery to commercialization will be described.

PS-2

The Manzanar Project: Contributions of In Vitro Biology, Tissue Engineering, Proteomics, and Beyond. GORDON SATO. Department of Fisheries, PO Box 18, Masawa, ERITREA. Email: imu@eol.com.er

In the 1960s, microbiological approaches and thinking were useful in sorting out issues such as dedifferentiation versus selection, and enrichment culture methods for obtaining cultures with differentiated function. Since the 1970s, the emphasis has shifted to using cultured cells to analyze problems of integrated physiology such as growth control, cancer, etc. Approaches to organizing research institutes, and the use of applied science to alleviate practical problems such as poverty and hunger will be discussed.

J-1

Studying Blood Vessel Development Using the Zebrafish. N. D. Lawson, A. M. Vogel, and B. M. WEINSTEIN. Laboratory of Molecular Genetics, NICHD, NIH, Bethesda, MD 20892.

The regulation of blood vessel formation is currently a subject of intense research study, with profound potential implications for human health. Antiangiogenic and proangiogenic therapies show enormous promise for combating cancer and treating limb or cardiac ischemia, respectively. Efforts to develop these therapies, as well as targeted treatments for atherosclerosis, depend on a detailed understanding of the genetic mechanisms of normal blood vessel formation and on the identification of new molecular therapeutic targets. The zebrafish, a small tropical freshwater fish, possesses a unique combination of features that make it particularly well suited for these goals. The fish is a genetically tractable vertebrate with a physically accessible, optically clear embryo that makes it possible to observe literally every blood vessel in the developing animal, using nothing more than a low-power dissecting microscope. To further exploit these basic advantages, our laboratory has developed a number of powerful new experimental and genetic tools for studying blood vessel development in this model organism. These include a three-dimensional confocal microangiography technique, a complete, staged description of the vascular anatomy of the zebrafish embryo and larva (interactive online atlas available at http://eclipse.nichd.nih.gov/nichd/lmg/redirect.html), transgenic zebrafish expressing fluorescent proteins and other genes in the vasculature, and mutations that cause vascular-specific patterning defects. We have used these and other tools to gain new insights into the mechanisms that guide formation of embryonic blood vessels. These insights include our recent discovery of a molecular pathway for arterial-venous differentiation involving the hedgehog, vegf, and notch signaling pathways. By examining hedgehog pathway mutants and experimentally manipulating hedgehog signaling in vivo, we have found that sonic hedgehog (shh) signaling is necessary for specification of the trunk dorsal aorta. In the absence of shh signaling, the dorsal aorta fails to form, while ectopic activation of shh signaling leads to apparent -arterialization" of venous endothelial cells. We have used similar genetic and experimental methods to activate or repress vegf and notch signaling in developing zebrafish embryos, allowing us to establish a hierarchical pathway for arterial specification and differentiation of vascular endothelial cells. Our findings underscore both the complexity of mechanisms guiding embryonic blood vessel formation and the power of the zebrafish for dissecting these mechanisms.

J-2

Differentiation of Rat Bone Marrow Cells into Hepatocytes in Culture. M. MIYAZAKI and N. Huh. Department of Cell Biology, Okayama University Graduate School of Medicine and Dentistry, Okayama 700–8558, Japan. Email: hiromiya@md.okayama-u.ac.jp

Stem cells are thought to be pluripotent cells that can be differentiated into a variety of cell or tissue types and are ideal resources of transplantation therapy. Recent studies revealed that bone marrow (BM) cells could develop into hepatocytes by in vivo transplantation under certain circumstances. However, the mechanism of BM cell differentiation into hepatocytes is still unclear. Differentiation and regeneration of cells are mainly controlled by growth factor(s) or cytokine(s). Hepatocyte growth factor (HGF), originally identified and cloned as a potent mitogen for hepatocytes, plays an essential role on the development and regeneration of the liver. In the present study, we examined cytological effects of HGF on adult rat BM cells in culture to elucidate the mechanism of differentiation of BM cells into hepatocytes. At first we demonstrated the presence of cells expressing alpha-fetoprotein (AFP) and the HGF receptor c-Met in BM cells derived from adult rats. When the BM cells were treated with HGF in vitro, a part of the cells differentiated to express albumin. Furthermore, these cells expressed cytokeratins 8 and 18 as well, which are typically expressed in normal adult hepatocytes. These findings indicate that BM cells include AFP-expressing progenitor cells that can be differentiated into hepatocytes by HGF in vitro. The present culture, therefore, can be a useful resource for cell transplantation therapy for liver diseases.

J-3

Molecular Target for the Regulation of Angiogenesis. YASUFUMI SATO. Department of Vascular Biology, Institute of Development, Aging and Cancer, Tohoku University, Sendai 980–8575, Japan. Email: y-sato@idac.tohoku.ac.jp

Mouse embryonic stem (ES) cell culture system is a useful tool for the analysis of developmental biology in vitro. To explore a novel regulator of vasculogenesis or angiogenesis in the vascular system, we isolated genes that were exclusively expressed during the differentiation of endothelial cells (ECs). Flk-1(+)/VE-cadherin(-) cells define EC progenitors and Flk-1(+)/VE-cadherin(+) cells define ECs. We selectively sorted out EC progenitors and ECs, and then isolated genes expressed during the differentiation of EC progenitors to ECs using PCR-coupled subtractive hybridization. Among the genes obtained, we identified one gene that was inducible by VEGF in the murine ECs. Analysis of the nucleotide and deduced amino acid sequences revealed that the protein was composed of 930 amino acids including a HEXXH(X)18E consensus sequence of the M1 aminopeptidase family and is thought to be a mouse orthologue of puromycin insensitive leucyl-specific aminopeptidase (mPILSAP). The recombinant protein hydrolyzed N-terminal leucyl and methionyl residues from synthetic substrates. Immunohistochemical analysis revealed that mPILSAP was expressed in ECs during postnatal angiogenesis. Specific elimination of mPILSAP expression by antisense oligodeoxynucleotide (AS-ODN) attenuated VEGF-stimulated proliferation, migration and network formation of ECs *in vitro* and angiogenesis *in vivo*. To further clarify the mechanisms how mPILSAP regulates the migration of ECs, we analyzed integrin function. Although AS-ODN did not affect the level of expression of integrin a2, a5, b1, b3 and b5 subunits, it inhibited the adhesion of ECs to vitronectin, fibronectin or type 1 collagen. Mn^{++}, an activator of integrins, restored this inhibition. These results suggest that mPILSAP plays a role in angiogenesis at least in part by the activation of integrins in ECs.

J-4

Human Mesenchymal Stem Cells Differentiate to Multiple Lineages. A. Mackay, C. Cobbs, R. Dodds, and M. PITTENGER. Osiris Therapeutics, Inc., Baltimore, MD 21231. Email: mpittenger@osiristx.com

Stem cells of different types are found in the adult organism. Bone marrow has been shown to be one source for human mesenchymal stem cells (hMSCs). The hMSCs can be isolated and culture-expanded in vitro, and methods have been developed to cause their differentiation. These adult stem cells can be induced to form different cell types in vitro, such as adipocytes, chondrocytes and osteocytes. The hMSCs also support blood cells, providing soluble factors that promote in vitro hematopoietic expansion and differentiation. The in vitro expansion and cryopreservation does not inhibit differentiation of the hMSCs. Even after extensive culture, these adult stem cells retain a normal karyotype and telomerase activity. Additional lineages may be obtained by in vivo implantation, for example, myocytic differentiation. Further, the degree of differentiation may be modulated by the in vivo environment. Thus, MSCs are valuable experimental tools for understanding the molecular and cellular events that cause cellular differentiation, in vitro and in vivo. For lineage differentiation in vitro, results show cell fate is profoundly influenced by the signals that are provided by basal nutrients and cell-cell interactions, as well as by growth factor and cytokine concentrations. These signaling events likely play important roles in tissue regeneration in vivo that involve hMSC differentiation.

J-7

Sustainable Production of Bioactive Compounds from Sponges: Primmorphs as Bioreactors. WERNER E. G. MÜLLER. Institut für Physiologische Chemie, Abteilung Angewandte Molekularbiologie, Universität, Duesbergweg 6, 55099 Mainz, GERMANY. E-mail: WMUELLER@mail.UNI-Mainz.DE

Sponges [phylum Porifera] are a rich source for the isolation of biologically active and pharmacologically valuable compounds with a high potential to become effective drugs for therapeutic use. However, until now, only one compound could be introduced into clinics because of the limited amounts of starting material available for extraction. To overcome this serious problem in line with the rules for a sustainable use of marine resources, the following routes can be followed; first, chemical synthesis, second, cultivation of sponges in the sea (mariculture), third, growth of sponge specimens in a bioreactor, and fourth, cultivation of sponge cells *in vitro* in a bioreactor. The main efforts to follow the latter strategy have been undertaken with the marine sponge *Suberites domuncula*. This species produces compounds which affect neuronal cells, such as quinolinic acid, a well-known neurotoxin and other phospholipids. A sponge cell culture was established after finding that single sponge cells require cell-cell contact in order to retain their telomerase activity, one prerequisite for continuous cell proliferation. The sponge cell culture system, the primmorphs, comprises proliferating cells which have the potency to differentiate. While improving the medium it was found that besides growth factors, certain ions (e.g., silicate or iron) are essential. In the presence of silicate, several genes required for the formation of the extracellular matrix are expressed (silicatein, collagen and myotrophin); Fe(+++) is essential for the synthesis of the spicules, as result of the expression of the ferritin-, septin- and scavengr receptor genes. Furthermore, high water current is required for growth and canal formation in the primmorphs. The primmorph system has already been successfully used for the production of pharmacologically useful, bioactive compounds, such as avarol or (2'-5')oligoadenylates. Future strategies to improve the sponge cell culture are discussed; these include the elucidation of those genes which control the *proliferation phase* and the *morphogenesis phase*, two developmental phases which the cells in primmorphs undergo. In addition, immortalization of sponge cells by transfection with new genomic DNA appears to be a promising way, since recent studies underscore the applicability of this technique for sponges. Supported by grants from the Bundesministerium für Bildung und Forschung (National Center of Competence BIOTEC-MARIN). Müller WEG, Brümmer F (1998) Patent application AZ 198 24 384 [30.05.1998]●●Müller WEG, Böhm M, Batel R, De Rosa S, Tommonaro G, Müller IM, Schröder HC (2000) Application of cell culture for the production of bioactive compounds from sponges: synthesis of avarol by primmorphs from *Dysidea avara*. J Nat Prod 63: 1077–1081●● Müller WEG, Wiens M, Batel R, Steffen R, Borojevic R, Custodio MR (1999) Establishment of a primary cell culture from a sponge: Primmorphs from *Suberites domuncula*. Marine Ecol Progr Ser 178. 205–219.

J-8

Sponge Cell Cultures: Progress in Medium Design. S. DE ROSA, C. Iodice and G. Tommonaro. Istituto di Chimica Biomolecolare, CNR, via Campi Flegrei, 34, 80078 Pozzuoli (Napoli), Italy. E-mail: sderosa@icmib.na.cnr.it

Marine organisms and in particular sponges are an exceptional reservoir of structurally unique natural products, several of which have shown a wide variety of biological activities. In spite of this plentiful source of interesting new bioactive products, only a few of them have reached the stage of commercial production, the restricted availability of larger quantities of a defined organism as starting material for extraction of the compound being one of the major reasons for the limited attractiveness of the marine secondary metabolites for commercial utilization. It was hypothesized that cells of marine sponges can be cultured, and that the cultured cells will continue to produce bioactive metabolites [1]. Recent studies demonstrated the ability of sponge cell cultures to produce secondary metabolites [2, 3]. Thus far, primary cell cultures have been obtained from several sponges, with a low cell density in the cultures. This low proliferation can be explained by the culture condition utilized and/or the experimental approach to establish the culture condition. The lack of in-depth knowledge of the nutritional requirements of marine sponges is one problem. The development in primary sponge cell culture of some Demosponge, and progress in the composition of nutrient medium will be discussed. This research was funded by European Commission (Project: SPONGE, contract number QLK3–1999–0672). References [1] Pomponi SA & Willoughby R (1994) In: *Sponges in Time and Space* pp. 395–400. [2] Andrade P, Willoughby R, Pomponi SA & Kerr RG (1999) *Tetrahedron Lett* 40:4775–4778. [3] Müller WEG, Böhm M, Batel R, De Rosa S, Tommonaro G, Müller J & Schröder HC (2000) *J Nat Prod* 63: 1077–1081.

J-9

In Vitro Cell Culture from Marine Sponges, a Promising System to Produce Bioactive Compounds. E. RICHELLE-MAURER, J.-Cl. Braekman, C. Devijver, H. Gaspar, R. Gomez, R. Tavares, G. Van de Vyver, and R. W. M. Van Soest. Université Libre de Bruxelles, Laboratoire de Physiologie Cellulaire, IBMM, CP300, 50 av. F.D. Roosevelt, B-1050 Brussels, Belgium. Email: emaurer@ulb.ac.be

Among the marine invertebrates, sponges are a source for a great variety of biologically active compounds that could be of interest for the pharmaceutical industry. A few synthetic analogues of such sponge compounds have already found their way into industry, but synthesis of many others appears to be cost prohibitive or even impossible. In vitro sponge cell cultures are considered to be one promising alternative to extensive collections in the field for the commercial production of the desired substances. In this context, three sponge species, *Agelas conifera, Haliclona vansoesti* and *Xestospongia muta*, have been investigated. Cell cultures were successfully achieved in natural or artificial seawater with the maintenance of significant amounts of the target compounds. Chemical analyses revealed that sponge cells were involved in the production of these compounds for *A. conifera* and *H. vansoesti* while prokaryotic symbionts were responsible in such production for *X. muta*. The ecological role of the target compounds and the factors that could modulate their synthesis are discussed with a view to the optimisation of their in vitro production.

J-10

In Vitro Gene Expression in Marine Sponge Cells Stimulated by Phytohemagglutinin. R. WILLOUGHBY and S. A. Pomponi. Division of Biomedical Marine Research, Harbor Branch Oceanographic Institution, Inc., Fort Pierce, FL.

The marine sponge *Axinella corrugata* is a model for cell culture development and is known to respond to phytohemagglutinin (PHA) stimulation in vitro. This study documented changes in gene expression related to PHA treatment of sponge primary cell cultures. The research employed a novel cross-species technique in which marine sponge cDNA was hybridized to commercially available microarrays of human gene sequences. Widespread specific hybridization was observed. A panel of 146 potentially regulated genes was derived through basic statistical methods. Results indicate that PHA effects proliferative and anti-apoptotic molecular changes in marine sponge cells. The innovative cross-species microarray technique was established as an effective tool for gene expression profiling in non-model organisms, and also provided strong evidence of close homology between many human and sponge gene sequences.

T-1

Practical Comparison of Methods Used to Measure Predictive Capacity of In Vitro Tests. LEON H. BRUNER. Gillette Medical Evaluation Laboratories, 37 A Street, Needham, MA 02492–9120. Email: Leon_Bruner@gillette.com

We have recently completed a series of studies designed to compare several measures of predictive capacity commonly used to assess the performance of new toxicity test methods (NTM). Computer simulations were used to generate data sets similar to those that might be obtained from a large validation study. The parameters used in the simulations were adjusted between runs to produce data sets that had progressively poorer fit of the data to a prediction model that defined the relationship between the NTM and a reference test method (RTM). The data sets were then analyzed using three measures of predictive capacity: the 95% prediction interval, the correlation coefficient and the contingent probability statistics (CPS), sensitivity (Se), specificity (Sp), positive predictive value (PPV) and negative predictive value (NPV). When the association between a RTM and NTM is random, high values for the CPS can be arbitrarily obtained depending on where the cut-offs demarcating positive vs. negative responses are set. Additionally, the sum of Se+Sp=1, and the PPV is equivalent to the prevalence. When the fit of the data to an underlying prediction model is improved, quantitative measurements, such as calculation of the 95% PI, provide the best differentiation of predictive capacity. The CPS are surprisingly insensitive to changes in the fit of data to a defined relationship between an RTM and an NTM. Lastly, the simulations show that Se and Sp vary considerably depending on the distribution of toxicity included in a reference set of test chemicals used to validate a test. The importance of these findings is that they call into question current interpretations of the meaning and utility of these performance measures. The results of future validation studies need to be evaluated in light of this new information.

T-2

Evaluating Test Methods by Their Estimated Performance. RODGER D. CURREN. Institute for In Vitro Sciences, Inc., 21 Firstfield Road, Suite 220, Gaithersburg, MD 20878. Email: rcurren@iivs.org

New toxicity tests, especially in vitro tests, are often used as screens; i.e. they are used to preliminarily divide a test set of chemicals into positive or negative subsets. One of two strategies can then be applied. Either 1) the negatives can be accepted as correct and the presumed positives evaluated in a second tier test, or 2) the positives can be accepted and the negatives evaluated in a second tier test. There has been a tendency to assume that validation does not have to be as stringent for a screen as it does for a stand-alone test because the screen is only a preliminary assessment. However, both approaches 1) and 2) above use screens to make some firm decisions; the first to classify a chemical as non-toxic and the second to classify the chemical as toxic. It is often thought that sensitivity (fraction of known positives correctly identified) is the most important criterion and that specificity (fraction of known negative materials correctly identified) has much less significance. It follows that sensitivity alone could be characterized by using predominately positive chemicals in a validation study. We present data to show that this reasoning can lead to major errors. In approach 1), choosing a tier I test with low specificity will result in a large number of false positive materials passing through to the more expensive or animal intensive tier two test, possibly negating the advantage of having two tiers. This is especially relevant when several mechanistically specific tests are combined into a tier I battery. In approach 2), a low specificity tier I test means that many useful chemicals may be discarded, without necessarily decreasing the prevalence of positives in the subset of negative chemicals identified by the screen. Finally we show that using predominantly positive test materials to validate a screen can result in a serious overestimation of the test's sensitivity. For any test to be useful as a screening test or as a stand-alone test, it must it must be both sensitive and specific.

T-3

Is My Assay Good Enough? Experience from a Real-World Validation. JACQUELINE A. SOUTHEE. Gillette Medical Evaluation Laboratories, 37 A. Street, Needham, MA 02492. Email: southeebruner@attbi.com

It is often stated that a new toxicity test (NTT) must provide a level of protection equivalent to or better than the reference test it is intended to replace. The purpose of this presentation is to describe an approach that can be used to assess the predictive capacity of an in vitro test developed to predict results from the Draize eye irritation test. The first step in this process is to define the capacity of the in vivo test to predict its own result. This is done by determining how well the eye irritation response measured in one laboratory is predicted by other independent laboratories. Once the performance parameters of the in vivo test are defined the next step in the process is to determine the predictive capacity of the in vitro test for the eye irritation response using the same performance measures. This presentation will provide an overview of steps we have taken to define the performance characteristics of the Draize test for use in the assessment of an in vitro cytotoxicity test based on transfected corneal epithelial cells. Although the data presented will focus on a single test, the process can be generally applied to many other toxicity tests serving as a databased approach for judging the adequacy of an in vitro test.

T-4

Lifespan Extension in Vascular Tissue Engineering. L. E. NIKLASON, J. A. McKee, M. J. Boyer. Department of Biomedical Engineering, Duke University, Durham, NC 27708. E-mail: nikla001@mc.duke.edu

In the United States, 1.4 million patients per year undergo operations requiring arterial prostheses. Approximately 100,000 patients per year require vascular bypass of small caliber arteries, but have no usable autologous artery or vein for grafting. Hence, there is a pressing need for autologous vessels to treat atherosclerotic disease. Arteries have been successfully engineered from neonatal human smooth muscle cells (SMC), and from porcine and bovine SMC. But these approaches have not yet been translated to the growth of human arteries suitable for clinical use. We hypothesized that the inability of non-neonatal SMC to form arteries in vitro may be due to their finite lifespan in culture. Telomerase (hTERT), a reverse transcriptase, has recently been shown to immortalize a number of human somatic cells. Here we report that retrovirally-mediated restoration of telomerase activity in SMC, isolated from a child donor, extended in vitro lifespan in the absence of any detectable transformed phenotypes. The hTERT-expressing SMC were phenotypically indistinguishable from normal SMC. hTERT SMC retained a normal hill and valley morphology and striated cell bodies beyond 100 population doublings (PD), comparable to normal SMC at early passage. Late passage hTERT SMC also exhibited a differentiated phenotype similar to control SMC, retaining expression of SMC-characteristic proteins such as calponin (intermediate differentiation marker), smooth muscle myosin heavy chains (SM-MHC; advanced differentiation marker), and tropoelastin (extracellular matrix protein). To determine the impact of hTERT in human vascular tissue engineering, blood vessels were cultured from SMC using a biomimetic system as previously described. The physical appearance of hTERT vessels was dramatically improved compared to control SMC vesels. Wall thickness of hTERT vessels was significantly greater than controls ($P < 0.01$), and was similar to that of native saphenous vein. hTERT vessels also had greater cellular density and significantly greater rupture strengths than control vessels (356 ± 64 mm Hg for hTERT, vs. 59 ± 55 mm Hg for controls, $P < 0.0005$). Thus, hTERT expression enabled culture of arteries that were architecturally and mechanically far superior to those derived from control SMC.

T-5

Engineering Human Tissue Models to Study Basement Membrane Biology. F. Andriani, S. Griffey, and J. A. GARLICK. SUNY at Stony Brook and LifeCell Corp. E-mail: JGARLICK@NOTES.CC.SUNYSB.EDU

Interactions between keratinocytes and extracellular matrix (ECM) proteins at the basement membrane zone maintain tissue integrity and modulate keratinocyte adhesion, proliferation, migration and gene expression. While it is known that both keratinocytes and fibroblasts synthesize basement membrane components, this has been studied primarily in monolayer cultures, where keratinocytes do not express their differentiated phenotype and signals from a structured ECM are not present. To overcome this limitation, we have optimized skin-like, organotypic cultures by combining the two components thought to be critical in epidermal normalization—dermal fibroblasts and structured basement membrane. This was accomplished by growing keratinocytes on an acellular, human dermal substrate (AlloDerm®) that was repopulated with human fibroblasts which had migrated into the dermis from an underlying contracted collagen gel. These well-structured, organotypic cultures allow us to ask how pre-existing basement membrane components and dermal fibroblasts direct the organization of a structured basement membrane and the concomitant normalization of epidermal phenotype. We have found that organotypic cultures grown with dermal fibroblasts on pre-existing basement membrane components demonstrated a high degree of tissue normalization and generation of a structured basement membrane. In contrast, keratinocyte grown in the absence of pre-existing basement membrane components were well-stratified, but did not form structured basement membrane and showed altered tissue architecture. Without dermal fibroblasts, basement membrane assembled at discrete initiation sites, as long as pre-existing basement membrane components were present. Furthermore, assembly of well-structured basement membrane was associated with the linear deposition of the hemidesmosomal, receptor-ligand pair, laminin 5 and $\alpha 6$ integrin and with the proteolytic processing of laminin 5. Sustained keratinocyte growth required structured basement membrane and dermal fibroblasts while normalized tissue differentiation was fibroblast-dependent. To further understand the role of individual basement membrane components on epidermal phenotype, we have modified these organotypic cultures by growing epidermal tissues on individual ECM proteins. To accomplish this, tissues were grown on polycarbonate membranes that were coated with pure ECM proteins and layered onto contracted collagen gels with dermal fibroblasts. We found that the presence of basement membrane components Collagen Type IV and laminin 1 supported keratinocyte growth and survival, while ECM components not found in basement membranes did not. Our studies indicate that epithelial and mesenchymal components play a coordinated role in the generation of structured basement membrane and in the regulation of normalized epithelial architecture, growth and differentiation in tissues which closely mimic human skin. These novel, human tissue models will be described and their use as tools for understanding epidermal and basement membrane biology will be discussed. (Supported by grants #DAMMD17-01-1-0688 from the US Army Medical Research and Materiel Command, #2RO1DE011250–06 from the National Institutes of Dental and Craniofacial Research and LifeCell, Inc.).

V-1

Microgravity Analog Culture as a Model for Lymphocyte Performance in Space. NEAL R. PELLIS, Diana Risin, and Alamelu Sundaresan. NASA Johnson Space Center, Houston, TX 77584. E-mail: NPellis@ems.nasa.gov

Several investigators have shown that human lymphocytes display diminished performance in microgravity. The infrequency of opportunities for space experiments has promoted the development of microgravity analog culture systems that parallel some of the aspects of cell cultures in microgravity. An example is the rotating cell culture system that randomizes the cell orientation to gravity and induces a terminal velocity fall of the cells through the culture fluid. In the analog culture, human lymphocytes display the loss of locomotion and activation observed in microgravity. The analog culture is used as a platform to investigate the underlying mechanism of the diminished performance observed in microgravity. Results suggest that the unloading of gravity alters the shape of the cell, interferes with signal transduction, and induces reversible and potentially irreversible effects. There are reapportionments of the isoforms of protein kinase C, accumulation of inactive phospholipase C gamma, and decreased phosphorylation of membrane proteins. These are likely consequences of an earlier lesion directly induced by the unloading in analog microgravity culture conditions. Supported by NASA Grant NRA-OLMSA-02 and NAG-5–4072.

V-4

Blood Cells and Space Biology. A. J. SYTKOWSKI. Laboratory for Cell and Molecular Biology, Beth Israel Deaconess Medical Center, Harvard Medical School, Boston, MA 02215. E-mail: asytkows@caregroup.harvard.edu

The binding of polypeptide hormones to their cell surface receptors results in a change in receptor structure, leading to activation and/or inhibition of receptor-associated enzymes and signaling molecules. The ultimate result of signaling is a change in gene expression leading to cell growth and differentiation. Several observations indicate that changes in gravitational forces (or changes in hydrodynamic shear stress that are observed under various gravitational conditions) can affect intracellular signaling pathways. Changes in gravitational force have been shown to affect hematopoietic cells. Studies have demonstrated a striking decrease in lymphocyte activation in cells exposed to microgravity. *In vitro* growth and differentiation of $CD34^+$ bone marrow progenitor cells are profoundly affected by microgravity. Myeloid cell expansion was far slower in microgravity aboard two Space Shuttle missions than on earth. Importantly, erythroid progenitor numbers actually declined by >83% in microgravity. Especially intriguing are studies that demonstrate an alteration in the expression of the cellular oncogene c-*myc* in cells exposed to hypergravity and simulated or true microgravity, since we have documented a role for c-*myc* in the signal transduction pathway of erythropoietin (Epo) and have shown that protein kinase C (PKC) is essential for c-*myc* activation and for the mitogenic action of Epo in erythrid progenitors. Gravitational effects on PKC have been shown clearly in other hematopoietic cell types. The differing specific gravities of PKC and the other interacting macromolecular and small molecule signaling components render them sensitive to gravitational and fluid dynamic changes. This is especially important in the intracellular milieu that is composed of "fluid", "rigid", and "flexible-but-tethered" architectural components. We hypothesize that gravity alters this intracellular architecture and modifies the interactions of subcellular components in subtle ways leading to different responses to external stimuli at differing gravitational states. This can have profound effects on cell growth and differentiation.

CP-1

Mechanobiologic Research Bioreactor. ANDREA GUIDI. Universita' Federico II Napoli, Italy. Email: oceanomare@inwind.it

Advances in scientific knowledge and biotechnological capabilities herald an exciting new betrothal between tissue engineering and space biology. In this alliance, microgravity may become a surprising, unconventional, and yet attractive venue for the generation of macroscopic tissue. The physical basis for these effects is thought to be due to the low-shear fluid environment that the cells and tissues encounter in real or simulated microgravity. By eliminating sedimentation, buoyancy and density driven convection, the cells can be grown in a relatively quiescent fluid environment, where interactions between cells can occur with minimal disruption. This would allow cell aggregates and tissues to form as in vivo, but also suggests that methods are required, by which the mechanical environment may be modified to control the development of engineered cell structures. Extensive research on the effect of mechanical stimuli on cell metabolism suggests that tissues may respond to mechanical stimulation via loading-induced flow of the interstitial fluids. During the culture, cells are subject to a flow of culture medium. Flow properties such as flow field, flow regime (e.g. turbulent or laminar), flow pattern (e.g. circular), entity and distribution of the shear stress acting on the cells greatly influence fundamental aspects of cell function, such as regulation and gene expression. This has been demonstrated for endothelial cells and significant research efforts are underway to elucidate these mechanisms in various other biological systems. Local fluid dynamics is also responsible of the mass transfer of nutrients and catabolites as well as oxygenation through the tissue. Most of the attempts to culture tissue-engineered constructs in vitro have utilized either stationary cultures or systems generating relatively small mechanical forces. For example, cartilage constructs have been cultured in sinner flasks under mixed or unmixed conditions, in simulated and in real microgravity. In these mixing studies, however, it is difficult to definitively quantify the effects of mixing-induced mechanical forces from those of convection-enhanced transport of nutrients to and of catabolites away from the cells. At the state of the art, the presence of a more controlled mechanical environment may be the condition required in order to study the biochemical and mechanical response of these biological systems. Such a controlled environment could lead to an advanced fluid dynamic design of the culture chamber that could both enhance the local mass transfer phenomena and match the needs of specific macroscopic mechanical effects in tissue development. The cell culturist needs an in vitro environment which is provided by a device known as 'bioreactor'. A bioreactor is generally a tool or device for generating products using a biological system. A current problem in tissue culturing technology is the unavailability of an effective Bioreactor for the in vitro cultivation of cells and explants. It has, in fact, proved extremely difficult to promote the high-density three-dimensional in vitro growth of human tissues that have been removed from the body and deprived of their normal in vivo vascular sources of nutrients and gas exchange. A variety of tissue explants can be maintained for a short period of time on a supportive collagen matrix surrounded by culture medium. But this system provides only limited mass transfer of nutrients and wastes through the tissue, and gravity-induced sedimentation prevents complete three-dimensional cell-cell and cell-matrix interactions. Several devices presently on the market have been used with only limited success since each has limitations, which restrict usefulness and versatility. Further, no Bioreactor or culture vessel is known that will allow for unimpeded growth of three dimensional cellular aggregates or tissue. The environment created on Earth within a clinostat or rotating vessel s often referred to as "simulated microgravity". Presently, we know that microweight does evoke a number of effects on cells, and a number of well-controlled experiments have shown that the absence of weight can have profound effects on fundamental biological processes active within the cell. All conditions of "weightlessness" result from a net sum of all forces present equalling zero, not from an absence of gravity. Currently two bioreactors for the generation of simulated microgravity have been developed: the rotating wall vessel (RWV) developed by NASA and the random positioning machine (RPM) developed by Fokker Space. Both are based on time averaging of the weight vector acting on the biological particles, which are suspended in the culture medium inside the bioreactor, based on the 'requirement' that the weight vector should act for at least seconds in a constant direction to generate an effect in cells. In the case of the clinostat, while it slowly rotates, the particles are strongly influenced by viscous drag and tend to rotate with fluid medium. The bioreactor is an excellent example of how the skills and resources of two distinctly different fields can complement each other. Microgravity can be used to enhance the formation of tissue like aggregates in specially designed bioreactors. Theoretical and experimental projects are under way to improve cell culture techniques using microgravity conditions experienced during space flights. Bioreactors usable under space flight conditions impose constructional principles which are different from those intended solely for ground applications. The Columbus Laboratory as part of the International Space Station (ISS) will be an evolving facility in low Earth orbit. Its mission is to support scientific, technological, and commercial activities in space. A goal of this research is to design a unique bioreactor for use sequentially from ground research to space research. One of the particularities of the simulated microgravity obtained through time averaging of the weigt vector is that by varying the rotational velocity the same results can be obtained with a different value of g. One of the first applications of this technique in space biology was in fact the Rotating Wall Vessel developed by NASA, and originally designed to protect cell culture from the high shear forces generated during the launch and the landing of the Space Shuttle. A Bioreactor that is used both for ground and flight experiments provides the additional benefit of isolating dependent variable of gravity. This continuity will provide a means to compare results to a control experiment.

CP-2

Modeled Microgravity Culture of Pancreatic Islets for Transplantation Improves Morphology and Function. L. RUTZKY[1], M. Kloc[1], S. Bilinski[2], T. Phan[1], H. Zhang[1], S. Katz[1], S. Stepkowski[1]. [1]Department of Surgery. The University of Texas Medical School-Houston, Houston, TX 77030 and [2]Institute of Zoology, Jagiellonian University, Crakow, Poland. Email: Lynne.P.Rutzky@uth.tmc.edu

We have investigated whether culture of mouse pancreatic islets in modeled microgravity decreases immunogenicity and maintains islet morphology and function. Freshly isolated islets from C57Bl/10 (H-2^b) mice or islets cultured in stationary dishes or in HARV rotating wall vessel bioreactors for 7 days were transplanted without immunosuppression under the kidney capsule of either syngeneic C57BL/10 or allogeneic C3H (H-2^k) streptozotocin-treated diabetic mice. Allogeneic (n=18) and syngeneic (n=31) islet transplants cultured in either dishes or bioreactors survived >100 days with euglycemia (P=0.001), as compared to fresh islet allogeneic transplants (mean survival time, MST=12±1.73 days, n=7). Nephrectomy of the transplanted kidney quickly caused hyperglycemia. Islet titration studies revealed that 250 fresh or dish-cultured, but only 30–120 bioreactor-cultured islets produced euglycemia. Glucose tolerance and static glucose stimulation tests showed improved bioreactor-cultured islet function. Immunostaining showed that fresh islets contain dendritic cells with strong Class II MHC. Dendritic cells disappeared during 7 days of culture in dishes and bioreactors, which was confirmed by immunostaining and TEM analysis. Dish-cultured islets showed signs of degeneration, while bioreactor-cultured islets had well preserved ultrastructural morphology. Only bioreactor islets were devoid of tight junctional complexes (determined by colloidal lanthanum staining) and developed large channels between islet surface and interior, improving islet morphology and function. We conclude that bioreactor culture conditions decrease islet immunogenicity, while significantly improving islet morphology and function. Supported by NAG8–1585.

CP-3

Calcium Signaling Effects of Modeled Microgravity in Human Lymphocytes: Cell Signaling in Lymphocyte Locomotion. A. SUNDARESAN, D. Risin, and N. R. Pellis. Universities Space Research Association and Biological Systems Office, Johnson Space Center, Houston, TX 77058. E-MAIL:asundare@ems.jsc.nasa.gov

Modeled microgravity affects specific targets in human lymphocytes affecting functions such as activation and locomotion. In experiments conducted on shuttle flights as well as ground based microgravity cell culture analogs, both these important cellular functions were detrimentally affected. In order to dissect cell signaling pathways affected in thes cells, human lymphocytes were treated with the phorbol ester PMA and the calcium ionophore, ionomycin. There were no synergistic effects between the two. However PMA could restore lost locomotion and activation by 87%. Ionomycin did not have any restorative effects. Hence, calcium pathways were possibly intact. To further confirm this levels of the IP3 receptor in modeled microgravity were measured and found to be similar to 1g levels. Levels of calcium independent PKC isoforms were found to be dow-regulated by more than 60% while the calcium dependent PKC alpha was not affected at the protein levels. This also indicated that MAP kinase pathways might be unaffected as well. Further studies will further unravel the efffects of microgravity on lymphocyte function and thus the immune response.

CP-4

Spheroid Self-Assembly of Well and Poorly Differentiated Prostate Cancer Cells. R. M. Enmon, K. C. OCONNOR, H. Song, D. J. Lacks, and D. K. Schwartz. Tulane University and Medical School, New Orleans, LA 70018; Memorial Sloan-Kettering Cancer Center, New York, NY 10021; University of Colorado, Boulder, CO 80309. Email: koc@tulane.edu

Multicellular spheroids have application to tissue regeneration and in vitro drug testing where they mimic the structure and differentiated function of intact tissue. In particular, spheroids of tumor cells resemble micrometastases in their intrinsic drug resistance. Our research group has developed a kinetic model of spheroid self-assembly based on the Smoluchowski collision theory that accounts for multiple size classes in one-cell increments. Using well (LNCaP) and poorly differentiated (DU 145 and PC 3) human prostate cancer cells, the biological relevance and predictive capacity of the model were assessed. Kinetic coefficients generated for 2 x 104 DU 145 cells/cm^2 accurately predicted aggregation kinetics at half this density. To assess biological relevance, aggregation rates were compared with diffusive and adhesive properties. Diffusion coefficients ranged from 5 to 90 microm^2/min for single LNCaP and PC 3 cells, respectively. Similar diffusivities were predicted by the persistent random walk model and Einstein relation, indicating a random rather than projectile motion. LNCaP cells were the most adhesive in our study with reduced cell shedding, 100% adhesion probability, and enhanced expression of E-cadherin and collagen IV. There was an increase in DU 145 cells staining positive for E-cadherin from nearly 20% of single cells to uniform staining across the surface of all aggregates; under 30% of PC 3 aggregates stained positive. Aggregation rates were more consistent with adhesive properties than with motilities, suggesting that aggregation in our study was reaction-controlled. Relative to other assays employed here, aggregation rates were more sensitive to phenotypic differences in cell lines and described size-dependent changes in aggregation at a finer resolution. In particular, model results suggest similar aggregation rates for two-dimensional DU 145 and PC 3 aggregates and upwards of 4-fold higher rates for larger three-dimensional DU 145 spheroids, consistent with expression of adhesion molecules. The kinetic model should be useful in predicting spheroid size distribution to produce more viable cultures and as an assay to access the effects of novel drugs on cell adhesion.

CP-5

Premature Senescence or Cell Death: Cell Cycle Checkpoints Determine Cellular Response of Normal Human Fibroblasts to Oxidants. Q. M. CHEN, T. K. Dilley, V. C. Tu, and S. Purdom. Department of Pharmacology, University of Arizona, Tucson, AZ 85724. Email: QCHEN@EMAIL.ARIZONA.EDU

Early studies by Hayflick and associates found that normal human diploid fibroblasts (HDFs) undergo replicative senescence inevitably as a result of continuous passage in tissue culture. Although the limited replicative life span of HDFs is thought to relate to the process of aging or the mechanism of tumor suppression, the underlying cause of replicative senescence has remained unsolved. Reducing ambient oxygen tension or addition of antioxidant spin trap N-t-butyl-phenylnitrone (PBN) to culture media can extend the replicative life span of HDFs, suggesting a role of oxidative stress in replicative senescence. With early passage HDFs, mild doses of H_2O_2 convert quiescent cells to a phenotype indistinguishable from replicative senescent cells. This premature senescent phenotype is characterized by prolonged G1 arrest, elevation of $p21^{WAF-1/Cip1/Sdi1}$, inability to phosphorylate Rb, activation of neutral β-galactosidase, expression of multiple senescence-associated genes and enlargement of cell size without significant shortening of telomeres. In contrast to this premature senescence inducible with cells distributed in the G1 phase of the cell cycle, S-phase cells undergo apoptosis in response to similar doses of H_2O_2. Induction of apoptosis is p53 dependent, involving activation of caspases. When p53 is inactivated by human papillomaviral E6 protein, HDFs undergo cell death associated with mitosis in response to H_2O_2. This mitosis-associated cell death develops with a delayed time course of caspase activation but is inhibited with bcl-2 expression. Our data suggests that HDFs contain multiple cell cycle chekpoints that prevent cell proliferation in the presence of oxidative damage.

CP-6

Impairment of Malignant Tumor Phenotypes by Electrolyzed Reduced Water. S. SHIRAHATA, Taichi Hara, Takaaki Komatsu, Takeki Hamasaki, Hirofumi Nogami, Kiichiro Teruya, Shinkatsu Morisawa*, Kazumichi Otsubo*, and Yoshinori Katakura. Department of Genetic Resources Technology, Faculty of Agriculture, Graduate School of Kyushu University, Hakozaki, Fukuoka 812–8581, Japan. *Nihon Trim Co. Ltd., Oyodonaka, Osaka 531–0076, Japan. Email: sirahata@grt.kyushu-u.ac.jp

Daily intake of hydrogen-rich electrolyzed reduced water (ERW), which is produced near the cathode during electrolysis, has been reported to be beneficial for our health. We have reported that ERW scavenged reactive oxygen species (ROS) in vitro and protected DNA from oxidative damage (BBRC, 234, 269–274, 1997). Here we report that ERW contained 1–10nm of metal microclusters, which acted as active hydrogen (atomic hydrogen) donors and scavenged ROS. ERW scavenged intracellular ROS and changed the tumor phenotypes of cancer cells. Human fibrosarcoma HT-1080 cells exhibit malignant tumor phenotypes such as invasion, metastasis and angiogenesis. ERW did not significantly suppress the growth of HT-1080 cells, but suppressed the colony formation ability of HT-1080 cells in soft agar. ERW significantly suppressed invasion potency of HT-1080, which was determined by the matrigel invasion assay. ERW suppressed the secretion and expression of matrix metalloprotease (MMP)-2 and−9, which were related to tumor metastasis. The expression of TIMP-1 and−2, intracellular inhibitors of MMPs, was not affected by ERW. ERW also inhibited the activation of MMP-2, which is known to play an important role in metastasis. ERW suppressed the expression of the VEGF gene in HT-1080 cells. The conditioned medium of HT-1080 cells stimulated the angiogenesis in vitro, however, that of HT-1080 cell cultured in medium containing ERW suppressed the angiogensis. These results suggested that ERW scavenged intracellular ROS and impaired malignant tumor phenotypes such as invation, matastasis and angiogenesis without causing severe cell damage.

CP-7

Effect of Antitumor Activity of *Sargassum siliquosum* on Breast Cancer Cell Line T47D. M. I. TAN1), Y.Sandraling1), A. Siddiq1), A. Barlian1), S. Haga2). 1)Department of Biology—Bandung Institute of Technology (ITB), Indonesia, zip 40132; 2)Department of Anatomy, Nara Medical University—Japan. Email: marsel@bi.itb.ac.id

Sargassum siliquosum is a brown algae, which can be found abundantly along the Indonesian coast. It was reported that many species of Sargassaceae especially from Japan, have an antitumor activity, but there are no data about *Sargassum siliquosum* and many other Indonesian species of Sargassaceae. The aim of our research is to know whether the extract of this algae has an antitumor activity to the breast cancer cell line and the effect of the extract to the expression of Ki-67 antigen as a marker for proliferation in cancer cells. A water extracted of *Sargassum siliquosum* was used. The inhibition concentration of the extract was observed on breast cancer cell lines, T47D. From the result of the experiment to know the inhibition concentration, it could be supposed that this algae has an antitumor activity. The IC_{50} of the extract is 45 mg/ml and IC_{10} is 15 mg/ml. For further experiment, we observed the effect of this extract to the exprssion of Ki-67 antigen at the transcription level. T47D cells were cultured and treated with the extract using 15 and 20 mg/ml for 48 hours. Total RNA from the cells was collected and mRNA for Ki-67 antigen was amplified using primer for that antigen by RT-PCR. The result showed that the expression of the Ki-67 antigen mRNA was reduced if the cells treated with the 20 mg/ml of extract. It would be concluded that the proliferation rate of the cells was decreased after treated with this concentration for 48 hours.

CP-8

Manipulation of stem cells from midguts of *Heliothis virescens* (Lepidoptera) Larvae. M. J. LOEB, E. A. Clark, and Huy Do. Insect Biocontrol Laboratory, U.S. Department of Agriculture, Beltsville MD 20705. E-mail: Loebm@ba.ars.usda.gov

Insect stem cells can serve as working models for differentiation and developmental studies. However, a paucity of studies on insect stem cells exist, perhaps because few insect cytokines and growth factors are known with which to manipulate them. A good source of insect stem cells is the midgut. In Lepidoptera, the larval midgut consists of a monolayer of mature epithelial cells arranged over a basal membrane. Between the epithelial cell bases and the basal membrane are loosely attached, spherical stem cells. When midguts are removed to medium, stem cells (and a few other types) migrate out, facilitating collection. Density gradient separation can yield a preparation consisting of about 95% stem cells. Viability of the stem cells is determined by proliferation in response to fat body cytokines, and differentiation in response to midgut differentiation cytokines. Unfortunately, resonse is lost in about 24 h when stem cells are maintained at 25 degrees C. At 4 degrees C, viability extends to approximately 7 days. Addition of small amounts of cell-free conditioned culture medium allows survival and response to differentiation factors for approximately 30 days. However, conditioned medium prevents response to proliferation cytokines. As the purity of the cell preparation has been increased by better handling, responsiveness to fat body factors and differentiation cytokines decreases. Much remains to be discovered about stem cell regulatory growth factors and cytokines. However, combinations of insect and vertebrate hormones and cytokines have facilitated some unusual transformations in the midgut stem cells.

CP-9

Effects of a Fat Body Extract on Larval Midgut Cells and Growth in Several Lepidoptera. KIM ELSEN[1], Marcia Loeb[2], and Guy Smagghe[1]. [1]Laboratory of Cellular Genetics, Department of Biology, Free University of Brussels, Brussels, Belgium and [2]Insect Biocontrol Laboratory, USDA-ARS, Beltsville, MD 20705. Email: kim.elsen@vub.ac.be

Oral application of a fat body extract (FBX) from pupae of the tobacco hornworm, *Manduca sexta*, caused mortality in last-instar larvae of the cotton leafworm, *Spodoptera littoralis*, with doses of 10 µl FBX. Larvae of the gypsy moth, *Lymantria dispar*, were also affected at doses of 200 µl FBX. In contrast, no effect was seen in tobacco budworm (*Heliothis virescens)* larvae even with doses up to 500 µl FBX. In *Spodoptera*, clear symptoms of stem cell hypertrophy and sloughing of the old gut were seen in sections of the larval midgut under the light microscope. This suggests an extreme proliferation of stem cells to make a new insect gut. This phenomenon may explain why intoxicated caterpillars halted feeding. In addition, the appearance of apoptosis was examined. Interestingly, symptoms of precocious apolysis were seen in treated *Spodoptera*. With the use of monoclonal antibody, we confirmed the presence of molting hormone receptor in midgut cells and epidermis. To elucidate the mode of action of FBX on cellular level, we established stem cell cultures from the larval midgut of *Lymantria*, and tested the efects of FBX alone and in combination with human Epidermal Growth Factor (EGF) and insect hormones like ecdysone and 20-hydroxyecdysone. Data discuss the mode of action of FBX that may be of use to control pest insects.

CP-10

The Synthesis of Heat Shock Proteins During Gonadal Sex Differentiation in Male and Female Green Turtle Cell Cultures. A. BARLIAN, S Sudarwati, L. A. Sutasurya, and H. Hayashi. Department of Biology, Institute Technology Bandung, Bandung 40132, West Java, Indonesia. E-mail: aang@bi.itb.ac.id

Green sea turtle, *Chelonia mydas*, is one of temperature-dependent sex determination (TSD) animals. The cell lines originated from differentiating male and female gonads have been established, and the cultures were maintained in appropriate temperature that will produce only male or female gonad. This *in vitro* model was used as a cellular model to study the mechanism of TSD. The aim of this research was to find whether male and female cultures has different heat shock proteins (hsp) and furthermore whether hsp play a role in gonadal sex differentiation in TSD animals, especially in *Chelonia mydas*. Male gonadal cell culture was obtained from differentiating male gonads and was maintained in 25° C, while female cell culture was obtained from differentiating female gonads and were maintained in 31° C. The male and female gonadal cell cultures that were grown in different temperatures were then lebelled for six hours with ^{35}S-methionine and used as control. Male gonadal cell cultures were shifted to 31° C for six hours while female gonadal cell cultures were shifted to 25° C for six hours, and newly synthesized proteins were analyzed. The result from autoradiography showed that both male and female gonada cells synthesized hsp 20 and hsp 70. Those hsps might play a role as molecular chaperons when the cells under stress. Hsp 90 was obviously synthesized by female gonadal cell culture. The presence of hsp 90 in female gonadal cells suggested that hsp 90 could involve in keeping the inactive stage of steroid receptor, the important receptor in steroidogenesis. Although it seems that hsp were not involved directly in the mechanism of TSD in green turtle, the presence of hsp and the ability of the turtle cell culture to synthesize hsp could give a new insight in cellular models.

VT-1000

Authenticity of Animal Cell Culture by PCR and DNA Sequencing Analysis. MERRY LIU, Seh-ching Lin, Abbas Vafai, Francisco Candal. Scientific Resource Program, Center for Disease Control and Prevention, Atlanta, GA 30333.

Polymerase chain reaction (PCR) and DNA sequence analysis were performed to determine the authenticity of animal cell cultures in a laboratory setting where a large number of cell lines of different species are routinely propagated. The study was designed to generate a PCR fragment pattern of each cell line and then examine the pattern to identify each cell line by its species of origin and detect cross contamination. The aldolase gene family was selected for PCR amplification, since the aldolase gene family is highly conserved in a wide range of animal and human species. Total of 36 cell lines from 14 different species were selected from our Cell Culture Repository. Cell free extracts were amplified and PCR products were analyzed by agarose gel electrophoresis. The PCR results indicated that amplified intron G of aldolase gene in each species showed a unique band profile on agarose gels which allowed differentiation among various species and that the results were highly reproducible. However, we found that related species, such as rat and mouse or human and primate, had similar band size. DNA sequence analysis substantiated these observations and allowed further differentiation of closely related cell lines within the species. The results demonstrated that PCR analysis of the aldolase gene coupled with DNA sequencing is a useful tool for authentication of cell lines and has additional potential use to identify inter-species cross contamination.

VT-1001

Identification System for Cross-Contamination in Cultured Cell Lines by Combined Methods with STR-PCR and Molecular Cytogenetics in JCRB. H. TANABE, R. Iizuka, M. Hojo, M. Kurematsu, Y. Takada, T. Masui, and H. Mizusawa. JCRB Cell Bank, Division of Genetics and Mutagenesis, National Institute of Health Sciences, Tokyo 158–8501, Japan. E-mail: tanabe@nihs.go.jp

We have established a system for identifying the individuality for human cell lines that enables us to detect the cross-contamination. Since 1999 we have applied STR-PCR method that is constituted by *GenePrint* PowerPlex1.2 System (Promega) with multiplex PCR amplification of 8 STR and amelogenin loci by using ABI PRISM 310 Genetic Analyzer. Genetic typing was carried out in each allele by Genotyper (Perkin-Elmer Applied Biosystems) to represent electropherograms. All the possible labelled alleles were defined into the specific progression by describing the presence or absence as 1 or 0, respectively. For quick and precise detection, we developed the comparing program that can calculate evaluation values (EVs), which are indicating the similarities of STR profiling patterns represented by the progressions comprised of 1 and 0 between the particular cell line and all the remained over 300 cell lines. STR profiling data are available through our web site (http://cellbank.nihs.go.jp/). For more detailed analyses, we combined this system with molecular cytogenetics (M-FISH) that can allow us to visualize the chromosomal rearrangements between the original and derivative cell lines on the different sites of STR profiling patterns. At present we have found at least 10 cases of cross-contamination by our own system. We will demonstrate the relationships between "STR mutations" and chromosomal rearrangements.

VT-1002

Extracellular Matrix Enhances the Survival of Cryopreserved Adherent Cells. L. H. CAMPBELL, Kristy Sarver, Michael J. Taylor, and Kelvin G. M. Brockbank. Organ Recovery Systems, Inc., Charleston, SC 29403. E-mail: lcampbell@organ-recovery.com

With the advancements in high throughput screening and mandates all over the world to reduce animal testing, we have conceptualized the development of a convenient cell-based system of cells cryopreserved in microtiter plates for testing in a wide variety of applications. The retention of cells and the effects of cryobiological conditions on the adherence of cells to the substrate are critical issues for the success of this concept. In this study we have compared cell viability and cell retention of bovine corneal endothelial cells (BCE) after freezing and thawing on tissue culture plastic (TCP) versus an extracellular matrix (ECM) organized and deposited by the cells. Under appropriate conditions BCE lay down a fully formed ECM in vitro, thus providing a suitable model to compare cryopreservation of cells attached to TCP either directly, or via an ECM. Cells were plated onto either TCP or a newly formed ECM and then exposed to increasing concentrations of the common intracellular cryoprotectant dimethyl sulfoxide (DMSO). In addition, the effect of four separate cooling rates (-0.2°C/min, −1.0°C/min, −10.0°C/min and a modified −1.0°C/min protocol) were also evaluated because it is well established in cryobiology that cooling rate is a primary determinant of cell survival after cryopreservation. After optimized thawing, cell viability and cell attachment were assayed using alamarBlue to assess metabolic function and Cyquant (DNA) assays to determine the recovery of cell numbers respectively. Interestingly, retention of cells was not markedly influenced by the nature of the respective substrates, but cell viability was significantly better for cells cryopreserved while attached to an ECM. Cooling rate was also shown to impact cell survival with lower recovery of cell numbers and cell viability after cooling at 10°C/min compared with the slower cooling regimens. Slow cooling (0.2 and 1°C/min) resulted in ~70% recovery of cells irrespective of the substrate, but viability was significantly better after cryopreservation on the ECM compared with TCP. For example, using slow cooling (0.2, or 1°C/min) survival was >50% for the ECM group compared with only 29% for the TCP group ($p = 0.001$). This preliminary study supports the notion that cells can be cryopreserved as adherent populations in microtiter plates with acceptable recovery of cell numbers and viability. Moreover, survival was influenced by the nature of the substrate suggesting that further optimization can be achieved by attention to the composition of the substrate.

VT-1003

Strategies For Improving The Cell Culture Medium Performance. VIJAI K. PASUPULETI*, Abbie More, and Natalie Savich. DMV International Nutritionals, 40196 State highway 10, Delhi, New York 13753. *Sai International, Geneva, IL 60134. E-mail: vijai@saiintl.com

Medium design plays a critical role especially in the manufacturing of monoclonal antibodies in view of the current manufacturing capacity constraints. The medium is typically designed based on the standard formulations and the scientific literature to achieve increased growth rates, higher cell density, secretion of the desired therapeutic proteins in larger quantities and no or minimal interference in downstream processing. All of these factors have a direct impact on the cost of the finished product, thus fermentation media plays a key role in controlling the cost of finished product. It is evident that no one medium works best for all the cell lines. This is because each cell is unique and demands different nutrients that works independently or synergistically to increase the yields and productivities. This paper reviews the strategies for improving cell culture medium performance: How a medium is selected: medium from the literature, medium constituent swapping, mass balance to determine elemental or molecular composition of the medium, changing one component and it's concentration keeping everything else constant, experimental design techniques, principles involved in design and optimization of media, role of media in product cost control in commercial cell culture applications. The application of non-animal protein hydrolysates in the cell culture media will be highlighted.

VT-1004

Multiple Defined Human Cell-Biomatrix Coculture Models for Tissue Growth and Differentiation Studies. R. K. SINGH, P. Collingsworth, V. Alapati, X. Chen, and G. P. Siegal. Diversified Scientific Inc., Birmingham, AL 35205 and Department of Pathology, University of Alabama at Birmingham, AL 35294. E-mail: SINGH@UAB.EDU

Amgel, a human biomatrix composed of the major extracellular proteins but generally free of growth factors (GF) has allowed the evaluation of phenotypic properties of human cells including motility and invasion. Importantly, Amgel's unique milieu supports proliferation of a variety of human non-transformed and transformed cell types. Based on these properties, we developed a number of defined cell-matrix bioassays that stimulate neovascularization in vitro, examining both early (mitogenic) and late (angiogenic) events. These include: 1) human vascular endothelial cells (HUVEC) cultured on 2-D biomatrix coatings of FGF-enriched Amgel in multiwell plates; 2) a human 3-D coculture system to study the angiogenesis cascade by employing Amgel microbeads (10–20 ml size) ± FGF using a rotary bioreactor for 1–10 days and 3) VEGF-secreting human cell lines used in an Amgel-embedded microvessel system. From the studies, we determined that HUVEC cocultured on Amgel exhibit mitogenesis but undergo differentiation only in the presence of exogenous specific GFs. Specifically, in the first model 5 mg/ml of Amgel was shown to actively support the growth of EC without inducing differentiation. Cocultures on FGF-formulated Amgel (60 ng/ml) stimulated sprout/capillary formation in a dose- and time-dependent manner. Control cultures on plastic or type I collagen-coatings showed poor EC growth (< 60% viability). In the second model, both EC proliferation (MTT assay) and morphogenesis (sprout/capillary formation) were quantified. An initial (days 1–5) mitogenic response paralleled the sustained release of FGF from the Amgel biomatrix. The physiologic nature of these in-vitro human cell matrix models should thus allow the dissection of important cellular and molecular signaling pathways responsible for tissue growth and differentiation.

VT-1005

Role of HGF/SF in Somite Myogenesis. M. SHIOZUKA, S. Yokoyama, and I. Kimura. Dept. of Basic Human Sci., Sch. of Human Sci., Waseda Univ. Japan. Email: shio@human.waseda.ac.jp

Hepatocyte growth factor (also known as scatter factor, HGF/SF) is a potent inducer of cell proliferation, dissociation and migration in diverse cell systems including myogenic cells. Our previous studies have demonstrated that HGF/SF appreciably promotes the dispersion and emigration of somitic myoblasts in vitro. The present study was carried out to assess the significance of HGF/SF by means of morphological, immunocytochemical and some biochemical methods using in-vitro model of somitic myoblasts motility. Our observations are revealing that HGF/SF and its receptor, c-Met were detectable in myogenic cells and that HGF/SF may act in an autocrine/paracrine fashion. In addition, the spatiotemporal distribution of HGF/SF protein in the developing chick embryo was examined by whole-mount immunohistochemical techniques. These results suggest possible role of this protein as a potent regulator of somite myogenesis.

VT-1006

Effects of Murine Bone Marrow Endothelial Cell Conditioned Medium on the Growth of Yolk Sac Hematopoietic Progenitors and Angioblasts. Q. R. WANG, X. D. Na, Q. Y. Xie. Research Laboratory of Blood Physiology, Central South University, Xiangya Medical School, Changsha, 410078, China. Email: qirwang@yahoo.com

In the present study, the effects of serum free murine bone marrow endothelial cell conditioned medium (mBMEC-CM) on the growth of yolk sac hematopoietic progenitors and angioblasts were investigated. Yolk sac cells were incubated in culture dishes. The adherent cells were cultured in 35-mm dish containing 1.5 ml DMEM with 15% FBS and 10% mBMEC-CM. Non-adherent cells were collected for semisolid culture assay of CFU-GM and HPP-CFC after being cultured in DMEM with 10% mBMEC-CM and 10% FBS for 24 hr. Colonies consisting of pure angioblasts were obtained in liquid culture system containing 15% FBS and 10% mBMEC-CM. The angioblasts were positive in phagocytosis and VIII Factor antigen. And they are round or oval sharp in morphology. mBMEC-CM could expand yolk sac hematopoietic progenitors in liquid culture system. The percentage of CFU-GM and HPP-CFC compared with the 0 hr control was 119.5%±5.7% and 130.8%±15.2% respectively after 24hr liquid culture ($p<0.05$). The percentage of CFU-GM and HPP-CFC compared with the 24 hr control was 158.6%±8.3% and 165.0%±19.4% respectively after 24hr liquid culture ($p<0.01$). The expansion effects of mBMEC-CM on CFU-GM and HPP-CFC were enhanced by compounding with FL and TPO. The percentage of CFU-GM and HPP-CFC compared with the 0 hr control was 132.0%±6.2% and 176.9%±22.1% respectively after 24hr liquid culture ($p<0.05$). The percentage of CFU-GM and HPP-CFC compared with the 24 hr control was 190.4%±8.9% and 227.5%±30.5% respectively ($p<0.01$). Conclusion: mBMEC-CM could support the growth of yolk sac angioblasts and hematopoitic progenitors. This promoting effect was further enhanced by addition of FL and TPO.

VT-1007

Bone Marrow Endothelial-cell-derived Factors Inhibiting the Growth of Bone-Marrow-Derived Fibroblast Colony-Forming Cells (CFU-F). B. H. WANG and Q. R. Wang. Research Laboratory of Physiology, Hunan Normal University, Changsha, Hunan 410081, P. R. China. Email: WBH@CS.HN.CN

The present study was intended to investigate the effects of the secreted factors of the bone marrow endothelial cells on bone-marrow-derived fibroblast colony-forming cells (CFU-F) for potential mechanisms upon which hematopoiesis may be regulated by them within the bone marrow microenvironment. After obtaining the serum-free conditioned media of the purified populations of human and murine bone marrow endothelial cells (hBMEC-CM and mBMEC-CM) in vitro, MW>10kD, 3–10kD and <3 kD components were sifted out from these media by means of serial ultrafiltration. Assays of CFU-F were performed to test the effects of BMEC-CM and their ultrafiltration-prepared components. The results showed that every one of hBMEC-CM, mBMEC-CM and their MW<3kD components exerted a suppressive influence on the proliferation of corresponding CFU-F but >10kD and 3~10kD components did not. The BMEC-CM and MW<3kD components decreased the number of CFU-F as well as the size of it. There were the markedly negative dose-dependent relations between the concentrations of MW<3kD component and the number of CFU-F. Our observatons suggest that the bone marrow endothelial cells can secrete at least a humoral factor (molecular weight less than 3000 Daltons) which has an inhibitory effect on the growth of CFU-F.

VT-1008

Dynamics of Epithelium Integrity by 3D Rendering of EGF/erb-B1 Complexes. B. KAEFFER[1], A. Trubuil[2], C. Kervrann[2], L. Pardini[1], and C. Cherbut[1]. [1]Institut National Recherche Agronomique, Unité Fonctions Digestives et Nutrition Humaine, France and [2]Laboratoire de Biométrie, INRA, Jouy-en-Josas, France. Email: kaeffer@nantes.inra.fr

Epithelial cells in the large intestine are organizing independent tubular structures maintained in fast renewal by a highly dynamic flow of informative molecules circulating extracellularly or through gap junction wiring. **Method:** We propose a microscopic multidimensional analytic system to provide quantitative recording of biomarker expression profiles along the entire living intestinal crypt or tissues maintained in primary culture. The system is amenable to semi-automatization by allowing a rapid extraction of intensities from image series of crypt cells multilabeled for nuclei, Epidermal Growth Factor receptors (EGF-R), gap junction proteins, mitochondria activities. **Results:** EGF peptide binding was demonstrated in colonic cells in crypt or primary culture along with gap junction structural protein by a recently designed 3 dimensional analysis software (Quant3D). Heterogeneity of EGF-R expression was very high along the crypt axis allowing the definition of membrane hot spots of EGF binding. These hot spots were related to the consumption of dextran sulfate sodium, a molecule used to induce microlesions along the digestive tract. Hot spots of EGF binding defined relatively to the stem cell level were maximum for cells situated below the senescent compartment. Rats submitted to nutritional stress were found to show the highest heterogeneity for the profiles of EGF-R labeling intensities. Endodermal structural proliferative units from primary cultures grown in rotating bioreactor for 5 to 40 days were found to contain 10 to 30 cells arranged according to a tubular symmetry. Profiles of EGF-R expression were relevant to the process of tissue reorganization. **Perspectives:** Our data confirm that 3D rendering of EGF binding is a rapid and relevant biomarker of crypt functioning and tissue reconstruction.

VT-1009

Phosphorylation of S146 and S153 at p21 by Protein Kinase C in Keratinocytes. M. KASHIWAGI[1] and T. KUROKI[2]. [1]Institute of Molecular Oncology, Showa University, Tokyo and [2]Gifu University, Gifu, JAPAN. Email: mariko@med.showa-u.ac.jp

We have been investigating molecular mechanisms underlying terminal differentiation of squamous epithelia. Among the 10 isoforms of Protein Kinase C (PKC), the eta isoform of PKC (PKCeta) is predominantly expressed in squamous cell epithelia and crucially involved in terminal differentiation of keratinocytes by inducing differentiation phenotypes and arresting the cell cycle at G1 phase. Our previous studies showed that PKCeta associated with the cyclin E/cdk2/p21 complex and inhibited the cdk2 kinase activity, leading to G1 arrest in normal human keratinocytes (NHK). In the course of this study, we found that PKCeta phosphorylated p21 in the cyclin E/cdk2/p21 complex. As shown by in vitro kinase assay, p21 at S146 was phosphorylated by PKCeta, while S146 and S153 were phosphorylated by PKCdelta. PKC-dependent phosphorylation of p21 at S146 prevents the complex formation of p21 with PCNA. Phosphorylation of p21 at S153 inhibits the nuclear localization of p21. In addition, expression of p21 protein was decreased in PKCeta overexpressing NHK cells. And cytoplasmic localization of p21 was observed in PKCeta overexpressing NHK cells. Our results suggest that the modulation of p21 function by PKC-mediated phosphorylation may regulate the terminal differentiation of keratinocyte.

VT-1010

RAD, A Small GTP Protein, is Identified as a Transdifferentiation Factor that Inhibits Mucous Cell Differentiation but Stimulates Squamous Cell Differentiation in Airway Epithelium. REEN WU, X. G. Shao, and M. J. Chang. Center for Comparative Respiratory Biology and Medicine, University of California, Davis, CA 95616. E-mail: rwu@ucdavis.edu

Squamous cell differentiation and mucous cell metaplasia are commonly found in conducting airway epithelium in response to injury and inflammation. The nature of the transdifferentiation of airway epithelial cells to a mucus secreting or a squamous type is not known. We have recently identified a Ras-like GTP protein, Rad, whose expression is inversely related to mucous cell differentiation. Using a Tet-ON inducible system, we demonstrated that an overexpression of Rad protein in cultured cells inhibted both mucin secretion and MUC gene expression, but it enhanced squamous cell differentiation. These effects would not be seen if a dominant negative (dn) mutant of Rad was overexpressed in cultured cells. The promoter-reporter gene expression analyses demonstrated that Rad affected the transcription of these differentiated marker genes of MUC5AC, MUC5B and small proline-rich protein (SPRR1B). These results suggest that Rad is an important transdifferentiation factor that regulates airway epithelial cell differentiation.

VT-1011

An In Vitro Model for the Rapid Screening of Potential Components and Formulations for Nasal Drug Delivery. E. SCOTTO-LAVINO, J. M. Easow, S. R. Simon, and E. J. Roemer. Department of Pathology, State University of New York at Stony Brook, New York 11794. E-mail: escottol@ic.sunysb.edu

Clinical trials have indicated that nasal delivery of a variety of drugs is both possible and, in some cases, superior to either injections or oral delivery. The two main drawbacks of nasal delivery are the limited maximum dose per spray and the rapidity of clearance from the nasal cavity. These difficulties can best be addressed early in the formulation development. Appropriate screening can eliminate early on those materials that do not penetrate rapidly enough to overcome the limited delivery time window (15–30 min) that results from nasal clearance mechanisms. Clinical trials, however, are both costly and time consuming, and are ill suited for screening large numbers of candidate formulations. Initial *in vitro* screening of drug formulation candidates can provide a rapid, highly effective and low cost alternative to expensive animal and human clinical screens. *In vitro* screening can also permit the evaluation of components and formulations that are not yet sufficiently refined for animal or human testing. When choosing an *in vitro* model it is imperative that the system be robust, reliable, and able to produce consistent, reliable data. We describe here studies evaluating MatTek's EpiAirway™ System as a pre-clinical screening model for nasal drug delivery formulations. The EpiAirway™ System closely resembles human nasal epithelium both structurally and functionally. The tissue construct consists of normal, non-smoking human-derived tracheal/bronchial epithelial cells (TBE) grown on collagen coated, porous cell culture inserts in serum-free medium to form a multilayered model that closely resembles the pseudostratified epithelium of the nasal cavity. The constucts have been demonstrated to be highly differentiated in culture, with numerous microvilli and cilia on the apical surface of the cultures as well as tight junctions. An apical air-interface design allows for both the introduction of test articles to a surface much like *in vivo* nasal mucosa, and measurement of transepithelial permeability and evaluation of trans-epithelial resistance (TER). The range and reproducibility of behavior of quadruplicate samples of EpiAirway tissue constructs was examined across 18 tissue lots. Tissues were evaluated both for structural integrity, by TER, and for viability, by MTT and LDH release. The TER of these model tissues is consistent with normal human airway tissue. Air-interface tissues, put through the mechanical manipulations of our drug studies, but not exposed to test articles retain TERs in excess of 300 ohms*cm^2 over the 3 hours of assay manipulations. The range of responses, as reflected in the variability within quadruplicate sets, is not unlike that which would be expected with *in vivo* subjects. These same samples retain full ability to reduce MTT, indicating robust and unperturbed metabolic function, and show no indication of stress in the form of dead and lysing cells, as illustrated by the low or undetectable release of LDH. Overall, the EpiAirway system exhibits a consistent profile of reliability with tissue responses that were remarkably reproducible in samples run on different days, by different technicians, using different tissue lots. While the system described here does not replace *in vivo* testing, it is a promising pre-clinical tool with great utility as a primary screen for the development of nasal drug delivery vehicles. Supported by RAIRE Grant No. STI9620074.

VT-1012

Evaluation of the Penetration and Cytotoxic Effects of Drug Formulations on an In Vitro Nasal Mucousal Model. J. M. EASOW, E. Scotto-Lavino, S. R. Simon, and E. J. Roemer. Department of Pathology, State University of New York at Stony Brook, Stony Brook, NY 11794–8691. Email: jeasow@ic.sunysb.edu

The nasal route is an attractive alternative to conventional oral or parenteral drug delivery. The nasal mucosa provides a moist and highly vascularized membrane, crucial to rapid absorption into the blood stream, thus facilitating faster transport to the site of action. The two main disadvantages of nasal delivery are the limited maximum dose per spray and the rapidity of clearance from the nasal cavity. These difficulties can best be addressed early in drug development. *In vitro* screening of drug formulations can provide an efficient and cost-effective method of identifying lead candidates prior to *in vivo* testing. We have evaluated the ability of a large number of drug formulations to penetrate mucous membranes using MatTek's EpiAirway™ System. The EpiAirway System consists of human derived tracheal/bronchial epithelial cells grown on a collagen coated membrane to form a highly differentiated, organotypic model with many of the same features of nasal mucosa. The cells produce tight junctions that inhibit the passage of low molecular weight solutes, as well as the flow of electric current, permitting the use of trans-epithelial resistance (TER) as a correlate of permeability. Concurrent evaluation of fomulation cytotoxicity is important in order to evaluate whether drug permeation is due to a significant loss of viability or if it is occurring on viable tissue constructs. For these studies two complementary systems: lactate dehydrogenase (LDH) release, to measure the accumulation of dead cells, and reduction of tetrazolium salt (MTT), to evaluate the metabolic activity of living cells; were used. EpiAirway cultures are obtained in phenol red and hydrocortisone free medium and are cultured at 37°C for 48 hours to allow the tissues to equilibrate and begin secreting mucin. Test formulations are applied to the apical surface at the air-tissue interface of quadruplicate tissues. Samples are drawn from the underlying culture medium at 15, 30, 60 and 120 minutes for assay of drug concentration and lactate dehydrogenase (LDH) evaluation of cell death. Following harvest of the final time points, TER of the cell constructs is measured to evaluate tissue integrity. Finally, the viability and relative metabolic activity of each sample tissue is evaluated by cell uptake and transformation of the formazan dye, MTT. To date we have studied and evaluated the effects of multiple nasal delivery test formulations for a variety of drugs. During the course of screening studies, several of the test articles were run more than once. In each case, the tissue responses were remarkably reliable and reproducible in samples run using different tissue lots. If test formulations incurred any cytotoxic effects, results were consistent across the multiple tissues dosed. Those formulations with evidence of cytotoxic results from LDH and MTT assay data also had significant reduction or loss of tissue resistance. However there were a number of test articles with reduced TER values and increased permeation presenting no cytoxic response. Overall, this model exhibited a consistent profile of reliability, responding with appropriate sensitivity to test conditions while retaining the characteristics expected of normal tissue and yielding reproducible data, which allowed for the rapid identification of lead candidates for pharmaceutical development. This study was supported by URECA grant #011145–1012218.

VT-1013

Early Markers of Cell Injury Following In Vitro Sulfur Mustard Exposure in Human Epidermal Keratinocytes. Offie E. Clark III, Eric W. Nealley, and WILLIAM J. SMITH. Biochemical Pharmacology Branch, US Army Medical Research Institute of Chemical Defense, Aberdeen Proving Ground, MD. E-mail: william.smith@amedd.army.mil

Sulfur Mustard (HD) is a potent alkylating agent. When exposed to human skin it will cause blister formation accompanied by epidermal cell necrosis. The death process is a compilation of many cellular activities overlaying each other. Following the initial chemical insult, the cell attempts to maintain homeostatsis. Eventually overwhelmed by the toxicant damage, the cell enters a controlled death cascade (apoptosis). With the depletion of energy stores, the cell deteriorates and succumbs to necrosis. Determination of the time frame and susceptibility of cells to HD injury is crucial to the development of therapeutic intervention. It has been postulated that the cells metabolic energy level plays a key role in the its ability to survive following HD exposure. Mitochondria are central to cellular metabolism. Fluorescence microscopy and flow cytometry were employed to monitor the loss of mitochondrial membrane potential following in vitro HD exposure. Using the fluorescent dye 5,5',6,6'tetrachloro-1,1',3,3' tetraethlybenzimidazolyl carbocyanine iodide (JC-1), we demonstrated that human epidermal keratinocytes (HEK) began to lose mitochondrial membrane potential in a dose-dependent manner (50–300 mM) within 4 hours following in vitro exposure to HD. This indicator of cell death preceded vital dye uptake by at least 6 hours. In this same 4 hour time period, HEK underwent extensive plasma membran blebbing, but these blebs were not apoptotic bodies since they contained no DNA. The earliest evidence of general caspase activation was occasionally, but not reproducibly, seen at 4 hours after HD exposure. Our data suggests that while HD may carry out its chemical reactivity quickly, there is a period of hours, possibly up to 4 hours, before biochemical activities culminate in death cascades. This may offer a window of opportunity for therapeutic intervention.

VT-1014

Preliminary Evaluation of a Cytotoxicity Model for Predicting Acute Oral Toxicity Test Starting Doses. G. Mun, R. Ruppalt, R. D. Curren, and J. W. HARBELL. Institute for In Vitro Sciences, Inc., Gaithersburg, MD, 20878. E-mail: jharbell@iivs.org

For almost fifty years, in vitro cytotoxicity methods have been studied as predictors of acute lethality in vivo. Recently Halle has compiled a Registry of Cytotoxicity (RC) which compares the LD50 (in mmol/kg; rat or mouse) of 347 chemicals with an average in vitro IC_{50} (in mmol/l; various cell types). A graph of the RC shows reasonably good correlation between lethality in vivo and cytotoxicity in vitro; 73 % of the points lie within a ± log(5) interval around the regression line. This has led Spielmann et al. to propose that, at a minimum, the results of in vitro basal cytotoxicity tests can be used to estimate the starting dose for in vivo LD_{50} tests. It has also been suggested that new cytotoxicity methods be first qualified for use in this scheme by testing them with RC chemicals having a range of toxicities and which fit closely to the RC regression line. To qualify, the new results should have a similar slope and lie within ± log(5) of the RC regression line. We investigated whether two commonly used cytotoxicity tests (neutral red uptake in BALB/c 3T3 [3T3] and normal human keratinocytes [NHK]) would qualify under these conditions. Eleven chemicals from the RC whose in vivo LD_{50}'s ranged from ~0.1 mmol/kg to ~100 mmol/kg were chosen for evaluation. All chemicals were tested under code using a well-defined standard protocol. Cytotoxicity in the treated cells relative to that of control cells was determined after 24 hr (3T3) or 48 hr (NHK) exposure. When the resulting data were overlaid on the original RC graph, all new data points lay within a ± log(5) interval around the RC regression line. The slopes of the regression lines for the new tests were 0.506 (3T3) and 0.498 (NHK) versus 0.625 for the RC. R^2 values were 0.985 (3T3) and 0.936 (NHK). We conclude that the proposed qualification criteria are reasonable and that both of these candidate tests have met those criteria.

VT-1015

Potential Use of J774A.1 Macrophage Cells to Biomonitor Estrogenic Activity in Non-Estrogen Dependent Tissue. Q. FELTY and D. Roy. Environmental Health Sciences, UAB School of Public Health, Birmingham, AL 35294–0022. Email: Qfelty@ms.soph.uab.edu

It is known that the immune system is modulated by sex hormones. Although macrophages are important in the immune system for phagocytosis, they also play a key role in cell signaling due to their ability to produce cytokines and reactive oxygen species. Macrophage cells are present in almost every type of tissue via systemic circulation. In contrast, there also exist modified macrophage cell types that are locally present in many tissues. The expression of aromatase in macrophages suggest that estrogen may be produced in these cells through the conversion of androgen to estrogen. We also know that estrogen receptors are present in macrophages, which suggest that estrogen may modulate the activity of these cells. Since macrophage cells are found throughout the body, these cells may mediate the effect of estrogen on non-estrogen responsive tissues. It is not clear whether the growth of macrophage cells is regulated by estrogen. In this study, we investigated the effects of synthetic estrogen, diethylstilbestrol (DES) and natural estrogen, 17-β-estradiol (E_2) on the growth of the J774A.1 mouse macrophage cell line. To determine the impact of DES and E_2 on cell growth, J774A.1 cells were subcultured in 48-well plates at a density of 8 x 10^3 cells/well in DMEM containing 10% fetal bovine serum (FBS) and allowed to grow until 40% confluent. Cells were then grown in serum free medium for 1 day. Following serum starvation, cells were grown in DMEM 5% charcoal-dextran stripped FBS and treated with estrogen. After 48 h the cells were treated with BrdU according to the instructions provided by the manufacturer. BrdU incorporation was measured using a spectrophotometer plate reader at dual wavelengths of 450 nm and 690 nm. We found that both DES and E_2 at concentrations ranging from 1 mg to 1 pg/ml caused a dose dependent increase in cell proliferation. Our results suggest that the growth of macrophage cells is estrogen sensitive, and an increase in macrophage cells in response to estrogen could occur in both estrogen sensitive and non-estrogen sensitive cells *in vivo*. The estrogen-responsive macrophage system could be used as a model to study the effects of sex hormones on non-estrogen sensitive tissues.

VT-1016

Regulation of Growth of Human Embryonic Kidney Cells Through a Novel Alu Sequence Repeat Containing Gene. K. P. SINGH and D. Roy. Department of Environmental Health Sciences, University of Alabama at Birmingham, Birmingham, AL 35294. E-mail: Kasingh@uab.edu

Wilms' tumor is an embryonic kidney malignancy in infants and children. Synthetic estrogen, diethylstilbestrol (DES) is a human carcinogen. In animal model, both natural and synthetic estrogen induces tumor. The role of estrogen in the Wilms' tumor development is not known. In this study we have tested the effect of DES on the human embryonic kidney (HEK) cells. Recently we have identified a novel gene containing Alu repeat sequence. The role of this gene in the cell growth/proliferation is not clear. HEK cells were grown to about 30-percentage confluence in the DMEM, F-12 medium supplemented with 10% fetal bovine serum. Cells were serum starved for 24 hrs and then treated with various concentrations (100, 10, 1, 0.1, 0.01, and 0.001 ng/ml) of DES. After 48 hrs of treatment, cells were counted. Total RNA was isolated from serum-starved cells. Results of the cell count revealed that 10 pg/ml of DES was able to induce significant cell proliferation by 40 percent as compared to the untreated control. Gene expression analysis by RT-PCR revealed lower expression of this gene in the serum-starved cells as compared to the cells grown in serum containing medium. The growth of human embryonic kidney cells was sensitive to the DES. These data suggest that estrogen may be involved in the induction of human embryonic kidney epithelial cells proliferation. The induction of this gene expression in the fast growing cells in the presence of serum indicates that this gene is involved in the regulation of HEK cell growth and proliferation.

VT-1017

Analysis of Cullin-5/VACM-1 mRNA Expression in Breast Epithelial Cells, Breast Cancer Cell Lines, and Both Normal and Tumor Tissues. M. J. FAY, G. A. Karathanasis, C. J. Mandernach, J. R. Leong, A. Hicks, K. Pherson, and A. Husain. Department of Pharmacology, Midwestern University, 555 31st St., Downers Grove, IL 60515. E-mail: mfayxx@midwestern.edu

Cullin-5/VACM-1 is a member of the cullin protein family. The major cellular function attributed to cullins is a role in the degradation of short-lived cellular proteins by the ubiquitin-mediated proteasome pathway. Since the region of the chromosome (11 q22–23) where cullin-5/VACM-1 is located is associated with loss of heterozygosity (LOH) in breast cancer, the cullin-5/VACM-1 gene product is a putative tumor suppressor. However, the expression and function(s) of cullin-5/VACM-1 in breast epithelial cells and breast cancer cell lines has not been extensively studied. The purpose of this research was to examine the expression of cullin-5/VACM-1 mRNA in breast epithelial cells, breast cancer cell lines, normal human tissues, and matched normal/tumor samples. Using RT-PCR and Northern blot analysis cullin-5/VACM-1 mRNA was expressed by primary breast epithelial cells, MCF-10A breast epithelial cells, and MCF-7 and MDA-MB-231 breast cancer cell lines. These same cells also expressed mRNA for other cullin family members (Cullins 1, 2, 3, 4A, and 4B) as determined by RT-PCR. A normal human tissue expression array revealed that cullin-5/VACM-1 mRNA is expressed in multiple human tissues with the highest expression being found in skeletal muscle and the lowest expression being found in the ovaries. A matched normal/tumor expression array revealed that there was differential expression of cullin-5/VACM-1 in some human tumors versus normal tissue. (Supported by Grant R15 CA85279 from the NCI)

VT-1018

Modulation of Fas Expression and Sensitivity to Fas-induced Apoptosis in Human Cancerous Pancreatic Ductal Cells Maintained in Culture and Xenografted in Nude Mice. SOROOSH RADFAR(1), Christian Davrinche(2), and Etienne Hollande(1). 1)Laboratoire de Biologie Cellulaire et Moléculaire des Epithéliums (EA-3032), Université Paul Sabatier, 38 rue des 36 Ponts, 31062 Toulouse Cedex, FRANCE and 2) INSERM Unité 395, CHU Purpan, BP 3028, 31024 Toulouse Cedex, FRANCE.

It is known that the cellular lines established from pancreatic tumors and maintained in culture, express Fas. However the results on Fas-induced apoptosis are contradictory. Some reports describe them as sensitive and others as resistant to Fas-induced apoptosis. We determined Fa's expression and sensitivity to Fas-induced apoptosis in Capan-1 cells. We followed the course of Fas expression and sensitivity to Fas-induced apoptosis in these cells maintained in culture and xenografted in nude mouse, during repeated passages of the cells from *in vitro* to *in vivo*. We studied the membrane expression of Fas by immunocytochemistry and by flow cytometry. We showed that the Capan-1 cells (between 30th and 90th passage) express Fas and that they are sensitive to Fas-induced apoptosis. In contrast, when these cells were xenografted in nude mouse, the membrane Fas expression was not detectable and the cells became resistant to Fas-induced apoptosis. These two characteristics reappeared gradually when the xenografted cells were returned to *in vitro*. The Fas re-expression in cells appeared to peak before the 10th passage. The alternation of Fas expression/non-expression was reproduced by successive passages of cells from *in vitro* to *in vivo* life (n=3). These results suggest the existence of modulation of Fas expression and sensitivity to Fas-induced apoptosis by environmental factors in the nude mouse. The contradictory data on Fas-iduced apoptosis sensitivity appear to stem from differences in the conditions of maintenance of cells

VT-1019

The Kinetics of Matrix Metalloproteinase Inhibition by Chemically Modified Tetracyclines. LISA C. CHEN, Wayne J. Bellucci, Sanford R. Simon, and Elizabeth J. Roemer. Department of Pathology, State University of New York at Stony Brook, Stony Brook, NY 11794–8691. E-mail: LCCHEN@IC.SUNYSB.EDU

Matrix metalloproteinases (MMPs) are a family of zinc dependent endopeptidases that are involved in the degradation and remodeling of the Extra Cellular Matrix (ECM). Tissue inhibitors of metalloproteinases (TIMPs) are inhibitors that regulate MMPs. However, when the protease/anti-protease balance is disturbed, diseases associated with the breakdown of ECM such as cystic fibrosis, arthritis, tumor invasion and metastasis may develop. Using a commercially available MMP-9 activity ELISA protocol, a group of synthetic inhibitors known as chemically modified tetracyclines (CMTs) were screened for their inhibitory efficacy. The assay was carried out using a 96 well plate coated with anti-MMP-9 antibody. Pro-MMP-9, the inactivated form of the MMP-9 enzyme, was incubated overnight to allow for antigen/antibody complex formation. Pro-MMP-9 was activated with 1 millimolar para-aminophenylmercuric acetate (APMA). Wells were washed using 5% HBSS/95% PBS followed by subsequent washes with 5% HBSS/95% PBS/0.05% Tween-20. CMT concentrations of 1, 5, 10, 15, and 25 micro molars were added to the wells. Following, an engineered pro-urokinase detection enzyme containing a sequence recognized specifically by MMP-9 and a chromogenic urokinase substrate were added to the wells. Cleavage of the urokinase substrate produced a color that was proportional to the amount of active MMP-9. Activity was determined by kinetic analysis at 405 nm. A K_i value measuring the strength of the enzyme/inhibitor complex was determined for each CMT, allowing for the categorization of our chemically modified tetracyclines as inhibiors for MMP-9. This can prove to be beneficial in determining which CMTs are potential candidates for possible anti-inflammatory disease management. Study supported by: NSF RAIRE Grant no. ST19620074, Howard Hughes Grant no. 711995–13605, USAMRMC (DAMD) 17-98-1-8560, NIH (DE-10985), Stony Brook Center for Biotechnology, Colla-Genex Corp.

VT-1020

The Effects of Chemically Modified Tetracyclines (CMTs) on MonoMac-6 Cell Secretion of Matrix Metalloproteinase-9 (MMP-9) and Tissue Inhibitor of MMP (TIMP). S. KHAN, S. R. Simon, and E. J. Roemer. Department of Pathology, State University of New York at Stony Brook, Stony Brook, NY 11790–8691. Email: soph7480@aol.com

MonoMac-6 cells are a human acute monocytic leukemia cell line. These cells are activated by various stimuli such as cytokines and phorbol myristate acetate (PMA), a phorbol ester. Activation produces an inflammatory response that results in the production and secretion of matrix metalloproteases (MMPs), specifically MMP 9 and MMP 2. MMPs are zinc endopeptidases involved in the degradation and reconstruction of the extracellular matrix in conjunction with tissue damage resulting from pathological conditions. The purpose of this study is to measure the proteolytic activity of the MonoMac-6 cells in the presence of PMA and/or chemically modified tetracyclines (CMTs). It has been shown previously, in *in vitro* studies that CMTs inhibit the degradation of the extracellular matrix in relation to MMPs. Specifically, this study is concerned with MMP 9 and MMP 2, which can be converted to active forms by compounds such as p-aminophenylmercuric acetate (APMA). However, MMPs can be inhibited by Tissue Inhibitors of Metalloproteases (TIMP). TIMPs inhibit the activity of all active MMPs and regulate the activation of pro-MMP by binding to it. MonoMac cells (1x10 6 cells/ml) ar placed in 5 ml of serum-free medium in T25 culture flasks. They are stimulated with 10 nM of PMA in the presence or absence of 10 uM CMT 300 and CMT 308. Samples are harvested at 24 hours and centrifuged to remove the cells. The supernatants are assayed by an activity ELISA (Amersham Pharmacia Biotech) to detect the total amount of MMP and TIMP secreted. APMA is added to the supernatants to activate all of the latent pro-MMPs, allowing for full quantitation by the MMP activity ELISA. The TIMP ELISA is capable of recognizing both free TIMP and MMP-bound TIMP. In addition, the samples are assayed by Gelatin Zymography using the Novex pre-cast gel system. Cells dosed with 10 uM CMT 300 showed inhibition of MMP 9 secretion, whereas 2.5 and 5 uM had no effect on MMP 9 levels. The level of TIMP-1 secretion by MonoMac-6 cells is upregulated in the presence of PMA and downregulated when CMT 300 is introduced to the cells in culture. Further studies will evaluate the role TIMP plays in disorders characterized by excessive MMP secretion. Supported by NSF RAIRE No. STI9620074, USAMRMC (DAMD 17-98-1-8560), NIH (DE-10985), Stony Brook Center for Biotechnology, and CollaGenex Corp.

VT-1021

Tumorigenicity and Toxicity Assessment of Thalidomide Alone and in Combination with Cisplatin in Mice and Cultured Murine Cells. S. K. MAJUMDAR and J. M. Ruddy. Department of Biology, Lafayette College, Easton, PA 18042. Email: Majumdas@lafayette.edu

Thalidomide's ability to inhibit angiogenesis has led to clinical trials determining its effectiveness at combating various types of cancer. This study explored thalidomide's antitumorigenic capabilities when administered alone (400 mg/kg every 2nd day for 15 doses) and in combination with the chemotherapy drug cisplatin (1.5 mg/kg every 4th day for a total of 4 doses) to DBA2/J mice whose solid tumors were induced by murine erythroleukemic cells (GM-86). Thalidomide treatment alone produced no significant effect on tumor development and metastasis. Mice that received both drugs had significantly lower instances of both primary and secondary tumors as compared to the untreated control group. Mice that received combination treatment developed significantly smaller tumors than the thalidomide treated group. Cisplatin, administered alone or in combination with thalidomide, led to significant delay in tumor formation and longer life span than was recorded in untreated mice. To further explore the action of cisplatin, *in vitro* cell multiplication studies were conducted using murine erythroleukemic cells (GM-86) and murine lymph node endothelial cells (SVEC4–10). The cells were treated with thaldomide (0, 30, 50, or 100 μg/ml), cisplatin (0, 0.1, 0.5, or 1.0 μg/ml), both (100μg/ml) thalidomide + 0.1, 0.5, or 1.0 μg/ml cisplatin, or neither. The cells were counted using the Trypan blue exclusion method at 24 hour intervals for 5 days. In both cell lines, thalidomide failed to inhibit cell proliferation, while cisplatin treatment, with or without thalidomide, significantly inhibited the multiplication in a dose dependent manner. We thank the Celgene Corporation for its generous gift of thalidomide.

JI-1000

Novel Insect Primary Culture Method by Using Newly Developed Media and Extra Cellular Matrix. S. IMANISHI[1], G. Akiduki[1], and M. Haga[2]. [1]Insect Biotechnology and Sericology Department, and [2]Insect Biomaterial and Technology Department, National Institute of Agrobiological Sciences, Tsukuba, Ibaraki 305–8634, Japan. E-mail: imanishi@affrc.go.jp

While over 500 insect cell lines have been established over the past 35 years, most were developed using undifferentiated, generalized tissues(such as embryos, or ovaries). Cell lines are very significant materials in molecular biology. However, cultural technique is not adequately established for most insect species and their tissues. And it takes over one year till establishment as a cultured cell line. Here we developed two types of novel culture medium and culture method. New medium, such as MX and SX, can easily increase cells migrated from several kinds of insect species and of tissues. MX medium is mainly better for Lepidoptera, Coleoptera, Hemiptera and their tissues. SX medium is better for the growth of Diptera's cells. Especially, MX30, including 30% volume of FBS in the MX medium, could accelerate a migration and multiplication of the cells from testis tissues of *Bombyx mori*, and could shorten the primary culture term for 2~3 months when used a small scale culture bed. Now we are continuing the primary culture of *Plautia stali* cells derived from embryo, of *Anomala cuprea* cells from fatty body, of *Agrius convolvuli* cells from fatty body, of *Bombyx mandarina* cells from fatty body and of *Bombyx mori* cells from fatty body, ovary, embryo, hemocytes and testis. *Culicoides oxystoma* cells from embryo are now growing in SX30 medium. Water soluble chitin, N-trimethylchitosan and Sulfonated that modified chitins extracted from pupa's skins of *Bombyx mori*, were good materials as extra cellular matrix. Especially, water soluble chitin promoted cell migration from gonad tissues, in a low density of 0.001%~1% W/V. These chitosans seemed to have a role to act as an adhesive between cell and culture bed. But chitosans have tissue specificity, i.e. fatty body tissue does not have a strong adhesive reaction with chitosans. A novel insect primary culture method by using newly developed medium and extra cellular matrix provides stimulatory factors to aid growth of the primary culture cells.

JI-1001

Development and Testing of Insect Cell Lines for Neuronal Characteristics. C. L.GOODMAN(1), A. Wang(2), A. H. McIntosh(1), H. Nabli(3), and J. Wittmeyer(3). (1)USDA, Agric. Res. Serv., Biological Control of Insects Research Laboratory, 1503 S. Providence Rd., Columbia, MO 65203; (2)Aventis CropScience, 2 T. W. Alexander Drive, P.O. Box 12014, RTP, NC 27709; and (3)Dept. Entomology, University of Missouri, Columbia, MO 65211. E-mail: goodmanc@missouri.edu

Few continuously replicating insect cell lines with neuronal characteristics have been identified to date. Cell lines with these characteristics can be used in physiological studies for understanding insect neuronal growth and development, as well as toxicity studies for evaluating biorational insecticides. The goal of this project was to develop and identify cell lines with neuronal characteristics for the latter use. Project objectives included: (1) the establishment of new cell cultures from insect nervous tissues; (2) the use of biochemical methods to screen newly and previously established cell lines for the presence of nervous tissue-specific proteins, including functional octopamine receptors. New cell lines were established from selected tissues of budworms, *Heliothis virescens*, and bollworms, *Helicoverpa zea*, including larval ventral nerve cords (4 lines) and midgut tissues (1 line), as well as embryonic tissues (10 lines). For characterization, immunocytochemical studies using an antibody to horseradish peroxidase (anti-HRP) were performed based on Jan and Jan's observation that anti-HRP specifically recognizes neuronal tissues (Proc. Natl. Acad. Sci. 79:2700, 1982). A total of 32 cell lines were subjected to immunostaining using an anti-HRP-FITC conjugate, with 11 cell lines being specifically recognized (including at least 1 neuronal line). Additionally, selected cell lines were tested for cAMP elevation in the presence of octopamine to identify those with functional octopamine receptors. Of the cell lines examined, 2 responded with statistically higher levels of cAMP when exposed to octopamine (i.e., including a new cell line from neuronal tissues of *H. zea* larvae).

JI-1002

Synthetic Activity of Cultured Corpora Cardiaca/Corpora Allata Complexes from the Two-Spotted Stinkbug, Perillus bioculatus. C. L. GOODMAN(1), R. M. Wagner(1), H. Nabli(2), D. Davis(1), S. Crimmins(1), and T. Okuda(3). (1)USDA, Agric. Res. Serv., Biological Control of Insects Research Laboratory, 1503 S. Providence Rd., Columbia, MO 65203; (2)Depart. Entomol., University of Missouri, Columbia, MO 65211; and (3)Dept. Insect Physiol. Behavior, National Institute of Sericultural and Entomological Science, 1–2, Ohwashi, Tsukuba, Ibaraki 305, Japan. E-mail: goodmanc@missouri.edu

Insect corpora allata (CA) are endocrine tissues which are globular in shape and are found behind the brain. These tissues produce juvenile hormone (JH), insect hormones (methyl epoxyfarnesoates) which are important in development and reproduction. The main objective of our study was to evaluate the synthetic activity of cultured CAs from the two-spotted stinkbug, *Perillus bioculatus*, with the eventual goal of characterizing the JH produced by this insect. Because of the closely integrated nature of the CAs with the corpora cordiaca (CC, neuroendocrine tissues), the CC was left attached to the CAs during the incubation. CC/CA complexes were incubated in MEM medium containing tritiated methionine for 2 hrs and the products elaborated into the medium were extracted and quantitated using a standard technique for measuring *in vitro* JH synthesis optimized by Feyereisen and Tobe (Anal. Biochem. 111:372, 1981). For initial characterization, thin layer chromatography was performed on the extracts using hexane and ethyl acetate (1:1) as the solvent system. These analyses indicated that primarily one major peak was present which migrated differently than those of JH I, II and III. Additionally, the effects of different concentrations of trans-trans-farnesol on the CA synthetic activities were determined, as well as the effects of the age and sex of the insects on these activities.

JT-1004

Toxicity of Alkylated Naphthalenes to a Rainbow Trout Gill Cell Line. V. R. DAYEH*, G. Jeremic*, S. Lee*, K. Schirmer*, P. V. Hodson**, and N. C. Bols*. * Department of Biology, University of Waterloo, Waterloo, ON, Canada, N2L 3G1. ** Department of Biology, Queen's University, Kingston, ON, Canada, K7L 3N6. E-mail: vrdayeh@sciborg.uwaterloo.ca

The toxic actions of 12 substituted naphthalenes were investigated with a gill cell line, RTgill-W1, from rainbow trout. These were 1-methyl, 2-methyl, and 10 dimethyls at positions: 1,2; 1,3; 1,4; 1,5; 1,6; 1,7; 1,8; 2,3; 2,6; 2,7. Cytotoxicity was measured using three fluorometric assays of cellular function. These were alamar Blue (AB) for metabolic activity, 5'-carboxyfluorescein diacetate-AM (CFDA-AM) for membrane integrity, and neutral red (NR) for lysosomal activity. The method of dosing the cell cultures influenced the detection of cytotoxicity. Little cytotoxicity was observed when the compounds, which were dissolved in dimethylsulfoxide (DMSO), were first mixed into medium followed by the addition of the mixed medium to cell cultures. However, cytotoxicity was observed consistently in nine of the compounds when they were dissolved in DMSO and the DMSO solution was added directly to the medium already within cell cultures. The difference is attributed to the retention of test compounds on vessel surfaces during mixing and to DMSO enhancing cellular uptake. The three cytotoxicity assays gave similar results. Two naphthalenes were not toxic, regardless of the dosing method. The substitution pattern appeared to influence toxicity, but substituted naphthalenes were not substantially more toxic than the parent compound, naphthalene.

JV-1003

Effect of Poly IC on Salmonid Cell Lines. S. J. DEWITTE-ORR*, L. E. J. Lee**, and N. C. Bols*. *Department of Biology, University of Waterloo, Waterloo, ON, N2L 3G1. **Department of Biology, Wilfrid Laurier University, Waterloo, ON, N2L 3C5. Email: sdewitteorr@yahoo.com

Viral infection is often associated with double-stranded RNA (dsRNA) because at some point during the replication of many viruses dsRNA is produced. Exposure of mammalian cells to poly IC (polyinosinic:polycytidylic acid), which is a synthetic dsRNA, has been shown to induce antiviral activities, inhibit normal and tumor cell growth, and cause apoptosis in selected cell types. The response of fish cells to dsRNA is less well known but such knowledge would contribute to understanding the evolution of responses and signaling pathways associated with dsRNA and the potential therapeutical application of poly IC in aquaculture. To this end, three salmonid cell lines were studied. These were macrophage-like RTS11 and fibroblast-like RTG-2 from rainbow trout and epithelial-like CHSE-214 from chinook salmon embryos. Exposure to poly IC in serum-free medium caused cell death after two weeks in RTS11 cultures but not in CHSE-214 and RTG-2 cultures. Death in poly IC-treated RTS11 cultures appeared to be by apoptosis: nuclear fragmentation was detected with H33258 staining and internucleosomal DNA fragmentation was found with DNA ladders by gel electrophoresis. Growth, which was measured as ^{3}H-thymidine incorporation, was inhibited by poly IC in RTG-2 but not CHSE-214. These cell lines should be useful for studying dsRNA responses and signal transduction pathways in salmonids.

I-1000

Maintenance of Midgut Epithelial Cells from *Dendroctonus valens* Larvae (Coleoptera: Scolytidae) In Vitro. L. SÁNCHEZ, J. L. Andrade, R. Cisneros, and G. Zúñiga. Laboratorio de Variación Biológica y Evolución, Escuela Nacional de Ciencias Biológicas-IPN, México, D.F. 11340. E-mail: lchapul@yahoo.com

As has been reported in a previous work, the culture of midgut epithelial cells from *D. valens* adult insects is possible. Although it has been established the culture conditions, improved cell adherence and made detailed observations of their morphology *in vitro* by scanning electron microscopy, it has been difficult to extent their life-span. It is well known that the rate of cell division and differentiation in larvae midgut is quite different to those observed in adult insects because of its different metabolic rate. So in order to determine the differences between larvae and adult midgut cells life-span in culture, the purpose of this work is to establish the adequate culture conditions to maintain larvae midgut epithelial cells *in vitro*. Midgut epithelial cells from second, third and fourth-instar *D. valens* larvae were grown between two glass coverslips placed at the bottom of each well in a 24-well multidish plate. Culture was incubated at 28°C (microaerophilic atmosphere) in RPMI 1640 medium supplemented with 10% fetal calf serum, 20-hydroxyecdysone and fat body extract from tenebrionidae *Soophobas morio* pupae. The pH of the medium was 6.3 and the osmolarity was 265 mOsm. The results shown that larvae midgut cells keep alive for more than 82 days. At least four kinds of morphologically different cells appeared in culture. Initially, spherical cells of various sizes were observed and seems to be stem cells, later appeared pear-shaped cells, spherical cells covered with microvilli and finally columnar-shape cells with central nucleus and microvilli at one end. All these cell types attached to the glass surface and did not present long cytoplamic projections to reach each other, they only remained together forming a sheet of cells like a monolayer that spread out on the surface of the coverslip. The percentage of confluence was 50%. Their cytoplasm was granulose as if the cells were in a high metabolic activity. In comparison to adult midgut cell culture, larvae cells were maintained for long time period, so these findings arises the possibility to obtain a primary cell culture of midgut epithelial cells from *Dendroctonus valens* in a future.

V-1000

Multifaceted Phosphofructokinase and the Apparent Pleiotypic Effect in Metabolic and Cell Cycle Control. MARCO RABINOVITZ. Biochemically Based Drug Design & Synthesis, 4504 Traymore Street, Bethesda, MD 20814–3965. Email: mrab@consultant.com

A broad range of observations in the literature, obtained from all levels of mammalian biological organization, including the intact animal, organ perfusion, cell culture, cell lysates and highly purified enzymes, can be integrated so that the pleiotypic effects of amino acid deficiency are explained by a single mechanism. The lesions in culture include inhibition of glycolysis, glucose uptake, and nucleic acid synthesis, and the disaggregation of cellular polyribosomes caused by a block in formation of the peptide chain initiation precursor, the 43S ribosomal subunit. This subunit contains the ternary complex, eIF-2:GTP:Met-tRNAi, whose GTP is hydrolyzed to GDP on peptide chain initiation, and requires replacement of the GDP with GTP for a new round of peptide synthesis to begin. The mechanism involves the inhibition of phosphofructokinase (PFK) by uncharged tRNA formed during amino acid deficiency and the resulting depletion of the enzyme's product, fructose-1,6-diphosphate. This diphosphate is an early precursor for the synthesis of phosphoribosylpyrophosphate (PRPP) and cofactor of eIF-2B, the guanine nucleotide exchange factor. PRPP is required for synthesis of nucleotides for RNA synthesis and eIF-2B is required for the exchange of GDP for GTP indicated above. Support for these observations are available for your inspection and indicate that a metabolic (rather than a genetic) process is the master driving force for nutritional, and possible other extracellular signals observed in culture.

V-1001

Distribution of Carbonic Anhydrase IV (CA IV) in Golgi Compartments of Polarized Human Pancreatic Duct Cells Expressing Wild Type or Mutated (ΔF508) CFTR (Cystic Fibrosis Transmembrane Conductance Regulator). C. SALVADOR-CARTIER, M. Fanjul, and E. Hollande. Laboratoire de Biologie Cellulaire et Moléculaire des Epithéliums (E.A. 3032), Université Paul Sabatier, 38, rue des 36 ponts, 31400 Toulouse, France. E-mail: hollande@lmtg.ups-tlse.fr

In the human pancreas, CA IV, a membrane-bound CA isoform, is found on apical plasma membrane of duct cells; it plays an important role in HCO_3^- secretion. This secretion is regulated by the Cl^- channel CFTR and is strongly disrupted in Cystic Fibrosis (CF). In human pancreatic duct cells expressing wild type CFTR, CA IV targeting to plasma membranes is polarization-dependent. In CF human pancreatic duct cells, the ΔF508 mutation of CFTR gives rises to disturbances of its traffic and a defective targeting of CA IV to the apical plasma membrane. We studied the distribution of CA IV in different compartments of mucus-secreting human cancerous pancreatic duct cells expressing wild type CFTR (Capan-1 line) or ΔF508 CFTR (CFPAC-1 line). Firstly, the polarized state of these two cell lines was established. In polarized cells, fluorescence reactions observed by confocal microscopy and immunogold staining confirm the presence of CA IV on apical plasma membranes and microvilli of Capan-1 cells but not on these membranes of CFPAC-1 cells. CA IV-intracellular compartments colabeling showed in polarized Capan-1 cells, a colocalization of CA IV with: calnexin, an ER marker ; ERGIC53, an ER-Golgi intermediate compartment marker ; 58K and Golgi zone, two Golgi markers ; γ-adaptin, a *trans*-Golgi network (TGN) marker and Muc5AC, a Golgi vesicle and secretory granule marker. Furthermore, treatment of Capan-1 cells with brefeldin A led to the disappearance of CA IV-immunoreactivity on apical plasma membranes. In polarized CFPAC-1 cells, a colocalization between CA IV and calnexin, ERGIC53, 58K, Golgi zone and γ-adaptin was observed. In contrast, we failed to observe any colocalization with Muc5AC. Our results suggest that, in wild type CFTR-expressing cells, CA IV is targeting to apical plasma membranes *via* Golgi compartments, whereas in ΔF508 CF cells, the traffic of CA IV may be disrupted at the exit site of the TGN. This may be due to an alteration in pH in this compartmnt or defective formation of Golgi vesicles.

V-1002

Morphological Survival of Cryopreserved Bovine Oocytes at Different Culture Periods After Thawing. L. SIMONETTI and M. R. Blanco. Facultad de Ciencias Agrarias, Universidad Nacional de Lomas de Zamora. Ruta 4 Km. 2 (1836), Llavallol, Buenos Aires, Argentina. Email: L_SIMONETTI@HOTMAIL.COM

Cryopreservation procedures commonly affect normal morphology of bovine oocytes. However, their morphological classification may be performed at different culture periods following thawing. Thus, the aim was to determine if time at evaluation would influence results. Oocytes (n = 1121) were collected and assigned to groups: I (Fresh oocytes), II (Frozen immature oocytes), and III (Frozen matured oocytes). Oocytes from II and III were submitted to slow freezing in 1.5 M ethyleneglycol before or after in vitro maduration (IVM) in a TCM199 base medium for 22 h at 39°C in 5% CO_2 in air, respectively. Incubation with sperm for in vitro fertilization (IVF) was performed in a TCM199 base medium for another 22 h in culture conditions. Assessment of normal morphology (NM) took place either following IVM or IVF for groups I (I-IVM and I-IVF, respectively) and II (II-IVM and II-IVF, respectively) and after IVF for all oocytes belonging to III. For such purpose, cumulus cells were removed and morphology was assessed by stereoscopic examination. NM was defined by observation of dark evenly granulated cytoplasm and regular shape. Data of morphology were expressed as percentages and analyzed by Chi-square. NM was not different between I-IVM (84.0) and I-IVF (85.4), however a lower rate for NM was achieved for II-IVF (51.4) when compared to II-IVM (27.2) ($P<0.05$), suggesting that culture period may affect results for NM of immature frozen oocytes, but not of fresh ones. Statistical differences were detected between fresh and immature or matured frozen oocytes regardless of time of evaluation (<0.05). Conversely, comparisons between frozen oocytes groups depended on time of evaluation, since when assessed after a similar incubation time following thawing, NM for II-IVM was higher than III (29.4) ($P<0.05$), while NM rates were not different between frozen oocytes submitted to the same incubation stage (II-IVF and III) ($P>0.05$). In conclusion, time at evaluation would influence morphological survival results of cryopreserved bovine oocytes.

V-1003

Morphology and In Vitro Fertilization of In Vitro Matured Bovine Oocytes After Cryopreservation Using BSA or FBS in Cryoprotective Solutions. L. SIMONETTI and M. R. Blanco. Facultad de Ciencias Agrarias, Universidad Nacional de Lomas de Zamora. Ruta 4 Km. 2 (1836), Llavallol, Buenos Aires, Argentina. Email: L_SIMONETTI@HOTMAIL.COM

Several factors may influence results of oocyte cryopreservation. The aim of this study was to asses morphological survival and *in vitro* fertilization (IVF) of *in vitro* matured (IVM) bovine oocytes after cryopreservation in solutions supplemented with BSA or FBS. A total of 575 oocytes were obtained from 2–6 mm size of follicles of abattoir-recovered ovaries. Oocytes were cultured for IVM in a TCM199 base medium for 22 h in culture conditions (39°C in 5% CO_2 in air) and assigned to groups: I (Control), II (CRYO-BSA), and III (CRYO-FBS). CPA (cryoprotective agents) consisting of 1.5 M ethyleneglycol (EG) in holding medium (PBS supplemented with 0.4% BSA or 10% FBS for groups II or III, respectively) were added in three steps at 24°C for 15 min and oocytes were conventionally cryopreserved. After thawing at 37°C, CPA were removed and oocytes were cultured for IVF in a TCM199 base medium containing a sperm concentration of 1 x 10^6, for 22 h in culture conditions. Then, cumulus cells were removed and morphology was assessed by stereoscopic examination. Normal morphology (NM) of oocytes was defined by observation of dark evenly granulated cytoplasm and regular shape. Oocytes having NM were fixed, stained and evaluated for the presence of two pronuclei as signs of IVF. Data were expressed as percentages and analyzed by Chi-square. NM for group I (90.9) was > II (23.0) and III (20.0) ($P<0.05$). Statistical differences for IVF were detected only between groups I (54.3) and III (28.8) ($P<0.05$), although a tendency towards a reduction of IVF for II (38.2) could be found when compared to the control ($P<0.10$). In conclusion, supplementation of the freezing medium with BSA rather than FBS would improve IVF of cryopreserved IVM bovine oocytes.

V-1004

Morphological Survival of Immature and In Vitro Matured Bovine Oocytes After Cryopreservation in Solutions Containing Different Protein Concentrations. M. R. BLANCO and L. Simonetti. Facultad de Ciencias Agrarias, Universidad Nacional de Lomas de Zamora, Ruta 4 Km. 2 (1836), Llavallol, Buenos Aires, Argentina. Email: L_SIMONETTI@HOTMAIL.COM

Both BSA and FBS are protein compounds commonly used in freezing solutions at 0.4% or 10% respectively, to achieve oocyte membrane stabilization. The objetive of this study was to determine morphological survival of immature (IMM) and *in vitro* matured (IVM) bovine oocytes after cryopreservation in solutions supplemented with standard or increased amounts of protein compounds. A total of 842 oocytes were collected and divided into five groups: fresh oocytes (A), IMM and IVM oocytes frozen in solutions supplemented with 0.4% BSA (B1 and C1) or 0.4% BSA plus 10% FBS (B2 and C2). Slow freezing in 1.5 M ethyleneglycol was performed before (B1 and B2) or after (C1 and C2) IVM in a TCM199 base medium for 22 h in culture conditions (39° C in 5% CO_2 in air). After thawing at 37° C, cryoprotectants were removed and oocytes were evaluated for normal morphology (NM) following IVM of A, B1 and B2, and IVF of all groups in a TCM199 base medium for 22 h in culture conditions. NM was defined by observation of dark evenly granulated cytoplasm and regular shape. Data were expressed in percentages and analyzed by Chi-square. NM for A (85.4) was greater than B1 (25.9), B2 (29.2), C1 (25.8) and C2 (34.4) ($P<0.05$). Statistical differences were not detected among all cryopreserved groups. In conclusion, additional supplementation over the standard of cryoprotective solutions with protein compounds failed to decrease susceptibility to membrane damage during th freezing process of bovine oocytes at different stages.

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P-1000

Transgenic Resistance in Wheat Containing Soilborne Wheat Mosaic Virus (SBWMV) Genes. HANBING AN, Sarita V. Elizabeth, and Jeanmarie Verchot-Lubicz, Department of Entomology & Plant Pathology, Oklahoma State University, Stillwater, OK 74075. E-mail: hanbingan@yahoo.com

Plant virus cause significant annual losses to crop plants. Soilborne Wheat Mosaic Virus (SBWMV) is agronomically important causing 80% reduction in yields in susceptible fields. Research efforts have focused on breeding for natural SBWMV resistance and novel methods to control SBWMV in the field have not been developed. For example wheat transformation offers an alternative technology to introduce novel genes to improve host defense against disease. In this study, wheat plants were stably transformed with the genes encoding the SBWMV coat protein (CP), movement protein (P37), and readthrough (RT) domain of the coat protein. The green fluorescent protein gene was fused to the viral CP, P37, RT genes and inserted into pAHC25 plasmids adjacent to the *ubi* promoter (pCP:GFP, pGFP:37, pGFP:RT) . One plasmid contains the p37 gene without GFP. Cultured immature Bobwhite embryos were bombarded with gold particles coated with each of the plasmids. We have regenerated a total of 78 transgenic lines bombarded with each pCP:GFP, p37, pGFP:37, pGFP:RT plasmids respectively. 20–30 transgenic plants of each line were inoculated with purified virus and classified as susceptible or resistant lines. Southern-blot analysis was used to verify 3 virus resistant lines were transgenic. For commercial production, identified transgenic plants that are virus resistant were crossed with commercial wheat varieties to produce marketable varieties of transgenic SBWMV resistant winter wheat. The mechanism of resistance was explored. These are the 1st transgenic wheat resistant to a soilborne pathogen.

P-1001

Engineering Marker-free Insect Resistant Transgenic Vegetables Using *Agrobacterium*. K. AZHAKANANDAM, V. A. Ansingkar, P. Girhepuje, M. Narendran, S. K. Mukherjee, and U. B. Zehr. Mahyco Life Sciences Research Centre, Dawalwadi, PO Box 76, Jalna 431 203, Maharashtra, INDIA. E-mail: kasi@lsrc.mahyco.com

Marker-free transformation systems have the advantages of introducing multiple agronomically important genes, and at the same time, avoiding the problem of introducing multiple copies of the selectable markers. Thus a system has been employed for the production of marker-free transgenic Tomato and Brinjal. Two different methods of *Agrobacterium* - mediated co-transformation were used: 1) a single *Agrobacterium tumefaciens* strain carrying two separate binary vectors [one vector carrying the *npt*II gene and the other vector carrying the *cry1A(c)* gene], 2) two different *Agrobacterium tumefaciens* strains each carrying a separate binary vector. This system has already been validated by us for Rice. A reproducible transformation system has been established for the production of insect resistant transgenic Tomato and Brinjal, as a prerequisite for generating a large number of marker-free transgenic vegetables. The stable integration and the expression of the introduced *cry1A(c)*, along with *npt*II marker gene in T0, T1 and T2 plants have been confirmed and characterized by PCR, Southern analysis, ELISA and Bioassay against a major pests, shoot and fruit borer of Brinjal (*Leucinodes orbonalis*) and fruit borer of Brinjal and Tomato (*Helicoverpa armigera*). The same system, with modification, has been employed to generate maker-free insect resistant vegetables.

P-1002

Development of Avocado (*Persea americana*) Leaf and Root Cell Suspension Cultures to Monitor Defense-responses. K. R. BOZAK-MIDDLETON and R. Li. Department of Biology, Cal Poly Pomona, CA 91768. E-mail: kbozak@csupomona.edu

We have determined that the ethylene produced during ripening of avocado fruit induces several genes including a cytochrome P450 designated CYP71A1, though its role has not yet been defined. As ethylene is also produced during wounding of plant tissue either by biotic or abiotic damage we discovered physical slicing of unripe fruit and roots also induces this gene. In order to study whether this gene is induced during pathogen infection by organisms such as the "black root rot" culprit, Phytophthora cinnamomi, we infected seedlings with fungal spores. Due to the unsynchronized cell response and general necrosis we developed an in vitro assay utilizing sterile cell suspensions derived from avocado leaves and roots. Cell suspensions from leaf tissue were found to double roughly every 7 days whereas root cells doubled more slowly (every 20 days). We are currently utilizing these cultures as a model system for pathogen infection using both fungal and chemical elicitors and will present our data on cytological changes, ethylene production and CYp71A1 expression.

P-1003

Field Tests to Assess the Impact of Transgene Encoded T4-lysozyme on *Rhizobium leguminosarum* bv. Viciae. INGE BROER. Universität Rostock, Justus v. Liebigweg 8; D-18051 Rostock, Germany. Email: Inge.Broer@biologie.uni-rostock.de

Transgenic potato plants expressing T4 lysozyme display enhanced resistance against the plant pathogen *Erwinia carotovora* (Düring et al., 1993). T4-lysozyme, as other lysozymes, is a bacteriolytic enzyme destroying bacterial cell walls. Hence, the transgene encoded T4-lysozyme may not exclusively harm bacterial pathogens, but also affect other soil bacteria necessary for plant growth and health. We therefore analysed the impact of T4-lysozyme in comparison to hen egg white lysozyme on symbiotic bacteria, using *Rhizobium leguminosarum* as a model organism. We could show, that growing in liquid medium, *R. leguminosarum* was extremely sensitive against both kinds of lysozymes. Additionally, the impact of crude extracts from transgenic potatoes on *R. leguminosarum* could be demonstrated. The sensitivity of *R. leguminosarum* was dependent on the number of cells present in the culture and was highest using a starting titer of 103bacteria. We therefore conclude, that *R. leguminosarum* is a suitable model organism to study the putative impact of transgene encoded T4-lysozyme on soil micro organisms (de Vries et al., 1998). Although, in the laboratory, the nodulation of the wild type macrosymbiont *Vicia hirsuta* was diminished by a pre-treatment of the Rhizobia with T4-lysozyme, no influence could be detected after the application on the root itself. Nevertheless, when T4-lysozyme was produced directly by transgenic *V. hirsuta* roots in sterile culture, the number of nodules was reduced to less then 50% (Broer et al., in prep.). In contrast to this, the nodulation of wild type roots grown in the direct neighbourhood of transgenic potatoes was not influenced by T4-lysozyme possibly excreted by the transgenic root neither in the greenhouse nor during a three-years field trail.

P-1004

Platform Technology for Engineering Pest Resistance in Vegetable Crucifers. Huaiyu Wang and DANIEL C. W. BROWN. Agriculture and Agri-Food Canada, Southern Crop Protection and Food Research Centre, 1391 Sandford Street, London, Ontario, Canada N5V 4T3; E-mail: browndc@em.agr.ca

Cabbage (*Brassica oleracea var. capitata*) is reputed to be recalcitrant to genetic transformation. We have developed a 3-step protocol optimization approach for *in vitro*-based research that allows a rapid and simultaneous optimization of multiple germplasm accessions. The approach was applied to the optimization of cabbage hybrid and inbred lines and subsequent transformation protocols. Although regeneration capacities of 20 target cabbage lines were low and differed widely in the first *in vitro* screening, the most influential factors affecting regeneration were identified and ranked. A second optimization focused on the four most important factors and regeneration frequencies of most cabbage lines were greatly improved: 75% the lines could be regenerated at a 70%+ regeneration frequency with 10+ shoots per hypocotyl explant. The poorest responding germplasm was subjected to a third optimization round and regeneration frequency was increased to 95% with 9.5 shoots per explant. A histological study on organ regeneration from cabbage hypocotyl explants showed the source of *de novo* shoot meristems is the cells adjacent to the vascular bundle with cell divisions found only 8 days after preculture. Optimization of the *Agrobacterium* inoculation method and timing resulted in the successful insertion and expression of a *cry*1Ac gene. Marker gene expression in different parts of transgenic cabbage plants was quantified by ELISA and bioassays with target insect larvae resulted in 100% mortality without perceivable leaf damage.

P-1005

Gene Profiling on Blighted and Healthy Citrus Plants. E. F. CARLOS[1], K. S. Derrick[2], G. Barthe[2], and G. A. Moore[1]. [1]Horticultural Science, University of Florida, Gainesville, FL and [2]CREC, University of Florida, Lake Alfred, FL. E-mail: ECARLOS@UFL.EDU

Citrus Blight is an important citrus disease affecting primarily yield in adult plants and compromising maintenance of entire commercial blocks. It is present in most citrus producing areas around the world except those with a Mediterranean climate. Blight is associated with rootstock choice and annual tree losses in Florida (USA) and São Paulo (Brazil) range normally from 4 to 6%, making Citrus Blight a very important production concern in both areas. At present the disease is of unknown etiology. Therefore, a cDNA subtractive method was used to study gene expression patterns in blighted and healthy plants. Roots of a blighted Rough Lemon (*Citrus jambhiri* Lush.) rootstock supporting a Valencia sweet orange (*Citrus sinensis* L. Osbeck) canopy were dug out of a sandy soil after the presence of the disease was diagnosed by typical visual symptoms, zinc accumulation in the trunk and immunoassays of a diagnostic protein. Samples from healthy plants were taken from the same block of trees. Total RNA was obtained and RT-PCR was performed using Clontech Smart cDNA procedures, which enrich for messenger RNA transcripts. Hybridizations were performed between split sets of cDNAs of healthy plants against blighted ones, and vice-versa using Clontech PCR-Select cDNA subtractive procedures. Differentially enriched cDNAs were cloned and probed on membrane arrays with P32 labeled cDNAs made from subtracted healthy and blighted samples. Selected clones from 'Healthy' and 'Blighted' libraries were sequenced and analyzed. The process was repeated twice, and among the results, sequences with high homology to metallothionein, putative transporters and chitinase genes were found, as were some of unknown function. The metallothionein result is interesting because Blight causes unbalance in the mineral status of affected plants, and this gene maybe involved in this process. Putative transporters were also interesting because they may be related to the stress that Blight causes in plants. Finally, chitinases may be involved in some plant defense mechanism. Northern analyses to further characterize expression of interesting genes are underway.

P-1006

Expression of glpA/B Operon in Transgenic Chloroplasts to Degrade Glyphosate. AMIT DHINGRA, Ana M. Bailey, and Henry Daniell. Department of Molecular Biology and Microbiology, University of Central Florida, 12722 Research Parkway, Orlando, FL 32826–3227. Email: daniell@mail.ucf.edu

Glyphosate is an effective non-selective herbicide that is completely degraded by soil microorganisms. Several plant species have been engineered for glyphosate tolerance by overexpression of the EPSPS gene via nuclear and chloroplast genomes. In *E. coli* it has been demonstrated that the expression of *glp*A/B operon enables the bacteria to withstand very high levels of glyphosate in the media (10.2 g/liter). The *glp*A gene encodes for a phosphotransferase, which phopsphorylates the herbicide and the second gene *glp*B cleaves it to form AMPA, a substrate metabolized by the C-P lyase. It has been well established in our lab that chloroplast genomes are ideal for single step multigene engineering without the drawbacks of gene silencing and position effects. Therefore in the present study an attempt has been made to express *glp*A/B operon in transgenic chloroplasts under the regulation of specific untranslated regions. Chloroplast integration of the transgene will be confirmed by PCR and Southern analyses. We expect to observe high-level tolerance against glyphosate in the transgenic plants. Due to maternal inheritance of the chloroplast genomes, concern of pollen mediated transgene dissemination will be minimized. An attempt would also be made to assess the potential of the *glp*A/B operon as a selectable marker for chloroplast transformation in tobacco. If successful, it will be employed for the transformation of other cereal crops in the future where the application of this technology is limited due to lack of appropriate selectable markers, besides other limiting factors.

P-1007

Genetically Engineered Resistance for Barley Yellow Dwarf Virus (BYDV) Resistance in Wheat. P. DUPRE(1–2), M. Henry(1), A. Pellegrineschi(1), M. Trottet(2) and E. Jacquot(2). (1) International Maize and Wheat Improvement Center (CIMMYT), Apdo. Postal 6–641, 06600 Mexico D.F. (2) INRA Station de Pathologie Végétale, BP29, 35650 Le Rheu- France.

BYDV-PAV represents one of the most serious viruses of wheat and causes substantial losses throughout the world. In attempt to obtain BYDV resistant wheat, we genetically engineered this specie using the pathogen-derived resistance strategy. A total number of seven constructs harboring the BYDV-PAV sequences encoding for replicase, coat-protein, movement protein or a non-coding sequence corresponding to the promoter of sub-genomic 2 RNA were obtained. These sequences have been cloned into the unique *Bam*HI site of the $pAHC_{17}$ plasmid, in sense and in anti-sense orientation, between the UBI promoter and *Nos* terminator. Three wheat varieties were transformed by microprojectile bombardment with the constructs and the *bar* gene according to a CIMMYT protocol. Variable numbers of transgenic wheat were obtained for each construct–variety combination. For all transgenic plants, the number of copies and number of integration sites were obtained by Southern-blot on the T0 generation. BYDV resistance testing and transgene expression studies are currently being conducted on the T1 generation.

P-1008

Characterization of Citrus Plants Transformed with Genes for Resistance to Citrus Tristeza Virus (CTV). VICENTE FEBRES[1], Gloria A. Moore[1], and Richard Lee[2]. [1]Horticultural Sciences, Plant Molecular and Cellular Biology Program, University of Florida, Gainesville, FL 32611 and [2]University of Florida, CREC, Lake Alfred, FL. E-mail: VJFEBRES@MAIL.IFAS.UFL.EDU

A long-term solution to the problems of Citrus tristeza virus (CTV) and its vector, the brown citrus aphid is the production of citrus types that are genetically resistant or immune to the virus. Since such resistance may be achieved with a single gene, genetic engineering for CTV resistance via transformation is a viable option. Our laboratory has for some years been working on the *Agrobacterium*-mediated transformation of citrus plants with genes and sequences derived from CTV. We have used capsid protein (CP) sequences from mild (T30), quick decline (T36) and stem pitting (B249) isolates; a non translatable CP derived from T36; the minor CP gene (p27) from T36; the RNA dependent RNA polymerase (RdRp) from T36; the 3' end viral sequence in sense (S) and antisense (AS) orientation from a T36-like isolate from Florida; and a non-structural viral protein (p20). Using our transformation protocol we have produced over 100 plants that have been verified as transgenic by PCR. Southern analysis results indicate that we have obtained stably transformed citrus lines with one to several copies of the transgene per genome. In addition, Western blot analysis indicates that the transgenes from some of these lines are expressed to detectable levels. We are in the process of challenging these transgenic plants with CTV using aphid transmission and grafting to determine their level of resistance, if any.

P-1009

In Vitro Studies of *Mycosphaerella fijiensis* Toxins on Mesophyll Cells Viability. M. A. Colmenares, C. A. GIMENEZ, and E. Quintero. Laboratorio de Biotecnología Vegetal de La Universidad del Zulia, Facultad Experimental de Ciencias, Maracaibo, Venezuela, E-mail: cagimenez68@hotmail.com

The main object of this research was to evaluate the possibilities of use mesophyll cells suspension to study plant pathogen interactions of *M. fijiensis* and *Musa* spp. plants. Cultivar Williams (AAA) susceptible to *M. fijiensis* infections, FHIA-02 (AAAA) resistant and Yangamby Km5 (AAA) immune to *M. fijiensis* infections serve as a reference cultivars. Toxins were extracted with Chloroform using 40 days liquid culture derivates of *M. fijiensis*. Isolation of mesophyll cells were performed with 3 g of leaves tissues from *Musa* spp. Leaves were chopped in MS and filtered through 50 m m mesh. Cell viability was estimate with Evans Blue in presence of different dilutions of toxins extracts. Percentage of viable cells were use to study resistance of mesophyll cells to *M. Fijiensis* toxins. Our results demonstrate that in resistant cultivar FHIA-02 (AAAA) and immune Yangamby Km5 (AAA), no differences in cell viability estimation with Evans Blue were detected in all toxins dilutions but in the susceptible cultivar Williams (AAA) toxins extract, strong accelerated cellular death in just 24 hours at very low toxins concentration (~ 60 ng/ml). These results demonstrate a correlations between resistance and toxins activation of cell death in mesophyll cell. Respect Yangamby Km5 no information was available respect mesophyll cells response and cytological studies described cell guard necrosis of stomata after *M. fijiensis* mycelium penetration. We supposed that mesophyll cells in Yangamby Km5 (AAA) could be susceptible to the toxins and the cell guard necrosis function as a barrier to fungus penetration. However, our results show that mesophyll cells of Yangamby Km5 (AAA) are resistant to *M. fijiensis* toxins. The mesophyll cell could be very useful for plant-pathogen studies specially in combinations with more sensible techniques as Flow Cytometer, fluorescence probes, etc. Future experiments of functional Flow Cytometer will be performed in other to reduce initial amounts of leaves tissue and make a more sensible test.

P-1010

Inducible Expression of GFP as a Tool to Study Protein Movement Across the Host-Broomrape (*Orobanche aegyptiaca* Pres.) Interface. N. HAMAMOUCH[1], R. Aly[2], C. L. Cramer[1], and J. H. Westwood[1]. [1]Dept. of Plant Pathology, Physiology, and Weed Science, Virginia Tech, Blacksburg, VA and [2]Agricultural Research Organization (ARO), News Ya'ar Res. Center, Ramat-Yishay, Israel. Email: westwood@vt.edu

Orobanche aegyptiaca (Egyptian broomrape) is a parasitic angiosperm that subsists on the root of many dicotyledenous plants. The parasite lacks photosynthetic capacity and thus is unable to develop independently of a host plant. *Orobanche* develops a haustorium and draws photosynthates and water from the host causing significant reduction in crop yield and quality (Sauerborn 1991). Understanding protein movement across the host/parasite interface is a very important step for developing strategies towards engineering resistant plants to *Orobanche*. The Green Fluorescent Protein has been widely used as a marker for monitoring gene expression and protein movement in living cells. Fusing *GFP* to *Orobanche* inducible promoter would allow monitoring of protein from host cells to *Orobanche* cells and thus providing useful information on plant-parasite interactions. The *GFP* gene was fused to *HMG2* promoter, and *Orobanche* inducible promoter (Westwood et al., 1998) that will allow *GFP* to be exclusively expressed at the site of the parasite ingress and be monitored for movement into *Orobanche* cells. Two routes for protein movement are being investigated. The *HMG2* promoter:GFP gene construct will allow us to study protein movement to Orobanche trough plasmodesmata while *HMG2:SP:GFP* will allow the GFP protein to inter the secretory pathway and be monitored for movement to Orobanche cells via the xylem. These genes constructs have been generated, subcloned into pBIB binary vector and successfully introduced into *Arabidopsis thaliana* (L.) and tobacco (Nicotina tabacum L.) using *Agrobacetrium*- mediated transformation. The incorporation of these genes into the plant genome has been confirmed by PCR. Plants expressing GFP were parasitized with broomrape seeds and are under analysis for GFP movement to broomrape cells using fluorescence microscopy. The information obtained will be provide insights on how protein move from host plant parasite in general and will be used specifically in our study to optimize the effectiveness of Sarcotoxin IA, a potent toxin to *Orobanche* (unpublished results), to defend the host and confront the invading parasite.

P-1011

Post-transcriptional Gene Silencing (PTGS) Results in PPV Resistance of Transgenic Plum Trees After Four Seasons of Growth in the Field. JEAN-MICHEL HILY[1], Tadeusz Malinowki[2], Michel Ravelonandro[1], and Ralph Scorza[3]. [1]UMR GDPP-Virologie, INRA, Bordeaux, France; [2]Research Institute of Pomology and Floriculture, Skierniewice, Poland; and [3]USDA-ARS Appalachian Fruit Research Station, Kearneysville, WV 25443. E-mail: jhily@afrs.ars.usda.gov

Plum pox virus (PPV) is one of the most devastating diseases of *Prunus* (stone fruit) species. PPV has been spreading world-wide since its first report of occurrence in Bulgaria early last century. Recent reports document its spread to Chile, Canada and the U.S. where eradication efforts are underway. Breeding for resistance is an important control strategy, but few sources of high-level resistance have been identified, and these are multigenic, requiring long-term breeding efforts for their incorporation into commercial cultivars. Transgene-based resistance offers a complementary approach to developing PPV-resistant stone fruit cultivars. If this strategy is to be successful for tree fruits, the long-term stability of transgene-based resistance under field conditions is critical. We have shown that plum clone C5 carrying the PPV coat protein (CP) gene was highly resistant to PPV in greenhouse graft-inoculation tests (Ravelonandro et al., Plant Dis. 81:1231–1235, 1997). Scorza et al (Transgenic Res. 1021–1029, 2001) demonstrated that the PPV-CP transgene in the C5 clone was specifically methylated, a hallmark of post-transcriptional gene silencing (PTGS), a plant defense that until the report of C5 had only been described in herbaceous species. Field tests of C5 and PPV-CP transgenic clones susceptible to PPV have been underway in Poland for the past 4 years. These trees have been challenged with PPV either by graft inoculation or by natural exposure to aphid vectors. DNA analysis after 4 years indicated that the PPV-CP transgene in C5 remains methylated. RNA analyses demonstrated low expression of the transgene and barely detectable levels of viral RNA in aphid and graft-inoculated plants. Susceptible graft-inoculated clones contained high levels of PPV RNA. These results indicate both the efficiency and stability of PTGS-mediated virus resistance in plum under field conditions.

P-1012

A Gene Encoding a Novel Antimicrobial Protein Discovered in Loblolly Pine. YINGHUA HUANG, Nili Jin, Alex Diner*, and C.G. Tauer. Department of Forestry, Oklahoma State University, Stillwater, OK 74078, *School of Forestry, University of Florida, USDA Forest Service, Gainesville, FL 32611-0410.

Genes for resistance to plant disease are present in diverse taxa of higher organisms and play an important role in host defense against microbial attack. We isolated a complementary DNA clone from a cDNA library of loblolly pine. This cDNA clone, named PtAMP, contains an entire coding region of a functional gene and shows the presence of a signal peptide at the amino terminus of the protein, suggesting that the protein is synthesized as a preprotein and transported to particular cellular locations. In order to analyze its antimicrobial activity, purified PTAMP protein was tested for inhibitory activity against a variety of microorganisms, including six fungal phytopathogens, five bacterial phytopathogens and baker's yeast. Antimicrobial assay data showed that this antimicrobial protein is strongly inhibitory to a broad group of microbial phytopathogens, including fungi and bacteria. However, the PtAMP protein is not toxic to plant cells. Furthermore, the gene for PtAMP was introduced into the tobacco genome via *Agrobacterium*-mediated transformation. Overexpression of the PtAMP protein in transgenic tobacco plants results in inhibition to microbial growth *in vitro* and *in planta*. The PtAMP appears to be a natural antimicrobial protein that protects host plants from endemic disease in natural populations.

P-1013

Development of Transgenics in Mungbean (*Vigna radiata* L. Wilczek) Resistant to Yellow Mosaic Virus, Bruchids, and Herbicide Phosphinothricin. PAWAN K. JAIWAL. Department of Biosciences, M. D. University, Rohtak-124001, India. E-mail: PKJAIWAL@YAHOO.COM

Mungbean is an important pulse crop, which is principally grown for its protein rich seeds and sprouts in many developing countries of South East Asia. Though India is its main producer, its production has not improved significantly during the last four decades due its prominent susceptibility to yellow mosaic virus (MYMV), bruchids (*Callosobruchus* species) and competition with weeds. None of the known cultivar is fully resistant to MYMV, bruchids and herbicides. Therefore, introduction of genes conferring resistance to these has emerged as an important approach. The VMYMV coat protein and antisense replicase, *Phaseolus* a—amylase inhibitor and *bar* genes for resistance to viruse, bruchids and herbicide phosphinothricin (PPT) respectively, have been introduced in mungbean using *Agrobacterium tumefaciens* and cotyledonary node explants. The shoots recovered from *Agrobacterium* inoculated explants on selection medium were rooted in the presence of kanamycin or PPT. The plantlets, which were found positive for *nptII* or *bar* gene by PCR, were grown to maturity to collect T_0 seeds. Integration of transgenes and their expression was confirmed by Southern blot analysis and protein/enzyme assays.

P-1014

Genetic Engineering of Cereal Crops for Pest and Disease Resistance. VASANT JANAKIRAMAN[1], Juliane S. Essig[1], Marcy L. Main[1], Ryan E. Adams[1], S. Muthukrishnan[2], Karl J. Kramer[2], and Harold N. Trick[1], [1]Department of Plant Pathology, [2]Department of Biochemistry, Kansas State University, Manhattan, KS 66506. E-mail: vasant@plantpath.ksu.edu

Introduction of Pathogenesis-related protein (PR) and insect chitinase genes is a promising strategy for producing disease and insect resistant wheat and corn. In this regard, the spring wheat cultivar "Bobwhite" has been genetically engineered with a rice chitinase gene (*chi*11) and a rice thaumatin-like protein gene (*tlp*). The synthetic chitinase gene from *Manduca sexta* (tobacco horn worm) (*msc*) has been introduced into corn. The constitutive expressions of these genes were driven by the maize ubiquitin promoter in wheat and the chimeric HBT promoter in corn. Immature wheat embryos cultured three to five days on induction medium were co-bombarded with pAHCG11 (ubiquitin/*chi*11:ubiquitin/*bar*) and pAHCTLP (ubiquitin/*tlp*:ubiquitin/*bar*). The immature maize embryogenic calli were co-bombarded with pSK1 (HBT/*msc*) and pAHC20 (ubiquitin/*bar*). The transgenic wheat and maize clones produced were selected on 5 mg/L glufosinate. After bombardment, the transgenic lines recovered from tissue culture were analyzed by PCR and Southern hybridization for the presence of *chi*11, *tlp*, *msc* and *bar* genes. The expression of the transgenes was confirmed by Northern blot analyses and reverse transcriptase-polymerase chain reaction (RT-PCR). PCR analysis of the T_0 and T_1 lines confirmed the presence of the transgenes. Southern blot analysis indicated multiple integrations of the transgenes as well as segregation of the transgenes in the T_1 generation. Northern blot analysis of the transgenic maize (T_0 and T_1) lines indicated the accumulation of 1.7 kb *msc* transcripts. Northern blot analysis of the transgenic wheat (T_1 and T_2) lines indicated the expression of 1.3 kb *tlp*, and 1.2 kb *chi*11 transcripts. The results of RT-PCR further reiterated the expression of the transgenes in the T_1 and T_2 generations. Bioassays on these transgenic plants will be discussed.

P-1015

The Occurrence of CMV-specific Short RNAs in Transgenic Tobacco Expressing Virus-derived Double-stranded RNA is Indicative of Resistance to the Virus. K. KALANTIDIS[1], S. Psaradakis[1], M. Tabler[1], and M. Tsagris[1,2]. [1]Foundation for Research and Technology—Hellas, Institute of Molecular Biology and Biotechnology, P.O. Box 1527, 71110 Heraklion, GREECE and [2]Biology Dept., University of Crete, 71110 Heraklion/Crete, GREECE. Email: kriton@imbb.forth.gr

Expression or introduction of ds RNA in eucaryotic cells can trigger sequence-specific gene silencing of transgenes, endogens and viruses. Transgenic plants producing double-strand RNAs with homology to viral sequences are likely to exhibit pathogen derived resistant to the virus. Cucumber mosaic virus (CMV), a very widespread virus with over 1000 host species has the natural ability to suppress silencing in order to establish infection. Here, we report the generation of transgenic tobacco lines resistant to CMV via the introduction of a DNA construct containing an inverted repeat of CMV cDNA. The construct leads to the transcription of an RNA able to form an intramolecular double-strand. The presence of the transgene was confirmed and transgenic plants were challenged with CMV. Three categories of plants could be discriminated: susceptible plants, which typically reacted with milder symptoms than the wild-type control, a 'recovery' phenotype, and plants that showed complete resistance. Northern analysis showed that the expression of CMV ds RNA caused in some transgenic lines the generation of short RNAs characteristic of PTGS. Those lines were CMV-resistant. The correlation between the detection of short RNAs and virus-resistance provides a molecular marker that allows to predict the success in attempts to engineer virus-resistance by ds RNA.

P-1016

Genetic Dissection of RPP5-activated Defense Responses in *Arabidopsis*. K. KAHN, J. D. Tedman, K. Monaghan, J. E. Parker, and J. D. G. Jones. Sainsbury Laboratory, John Innes Centre, Norwich NR4 7UH. E-mail: kahn@bbsrc.ac.uk

We are investigating how the race-specific resistance gene *RPP5*translates perception of downy mildew (*Peronospora parasitica*isolate Noco2) attack into effective defense. To identify the components that cooperate with *RPP5*to achieve disease resistance, we are isolating mutants that are defective in *RPP5*-function. We are especially interested in mutations that partially suppress *RPP5*-mediated defence, as these are likely to prove critical in dissecting the intricate signaling network(s) controlling resistance. Approximately 316,000 M2 EMS-mutagenized Landsberg-*erecta*-seedlings have been screened, yielding 32 partially susceptible (PS) mutants. Complementation testing of the first 13 PS mutants revealed six complementation groups, including two that may represent previously undescribed genes. For one of these, PS 138, we have localized the mutation to a 55 kbp region on the lower arm of Chromosome 2 and mutation detection analysis and complementation testing are underway. The remaining four PS complementation groups include the previously described *pad 4*(4 alleles), *rpr2/rar1*(1 allele), *sid2/eds16*(1 allele) and most likely *rpp5*(5 semi-dominant, partial-loss-of-function mutants). During this screen, we have also identified up to 72 mutants that completely suppress *RPP5*function; these mutants are completely susceptible to Noco2 attack. Analysis of the first 37 mutants in this class revealed that 28 are *rpp5*alleles, 2 *eds*alleles, 3 *rpr1/sgt1b*alleles, and 4 *rpr2/rar1*alleles.

P-1017

Induction and Suppression of Secondary Metabolism Relating to Stress Responses of *Glehnia littoralis* Cell Cultures. Y. KITAMURA[1], Y. Ozeki[2], A. Yamada[2], Y. Ito[2], A. Ishikawa[1], and M. Watanabe[1]. [1]School of Pharmaceutical Sciences, Nagasaki University, Nagasaki 852–8521, Japan and [2]Department of Biotechnology and Life Science, Faculty of Engineering, Tokyo University of Agriculture and Technology, Tokyo 184–8588, Japan. E-mail: k-yoshie@net.nagasaki-u.ac.jp

Furanocoumarin biosynthesis was induced in non-anthocyanin-producing (White) cells, but not in anthocyanin-producing (Violet) cells of *G. littoralis* suspension cultures by yeast extract treatment. Effect of yeast extract was pursued on *PAL* and *CHS* transcript accumulations, their enzyme activities, and anthocyanin/furanocoumarin productions throughout 48hs. *PAL* mRNA accumulation started in White cells after 1 h followed by increase of PAL activity after 4 h and furanocoumarin production after 6h. The transcript accumulation, the enzyme activity and furanocoumarin accumulation became maximal at 6h, 12h and 48h, respectively. In the case of Violet cells, accumulation of *PAL* and *CHS* transcripts, their enzyme activity, and anthocyanin accumulation, which were highly expressed in non-treated cells, were suppressed. Especially *CHS* transcription and the enzyme activity were transiently knocked down after 12h, resulting in decrease of anthocyanin accumulation. To determine whether different *PAL* or same *PAL* is responsible for anthocyanin and furanocoumarin productions, cDNAs for *PAL* as well as *CHS* were isolated from *G. littoralis* cells and their sequences were determined. The results showed that same *PAL* genes seemed to work on both biosynthetic pathways.

P-1018

Gene Pyramiding of Recombinant Antifungal Genes in Transgenic Pea (*Pisum sativum* L). H. KIESECKER*, A. Richter*, E. Ebmeyer**, H.-J. Jacobsen*. *Department of Molecular Genetics, University of Hannover, Herrenhäuserstr. 2, D-30419 Hannover, Germany and **Lochow-Petkus GmbH, Bollersener Weg 5, 29303 Bergen, Germany. E-mail:KIESECKER@LGM.UNI-HANNOVER.DE

The two most important properties of legumes are the nodulation and mycorrhization abilities. Legumes can sufficiently grow on marginal soils in developing countries as well as under intensive farming conditions. In the past, diseases were difficult to avoid by the traditional way of extensive farming in developing countries, but due to the irresistible intensification of agriculture especially fungal diseases are increasing dramatically. Prophylactic or in response to a pathogen attack, plants can activate genes that produce enzymes involved in combating fungal pathogens. Subject of our work is a biotechnological approach for the resistance improvement of pea varieties against fungal diseases by pyramiding of recombinant antifungal genes via crossing. Based on an *Agrobacterium tumefaciens* mediated gene transfer system (Schroeder et al., 1993) we have succeeded in transferring agronomically relevant European pea varieties with five recombinant antifungal genes,namely chitinase from *Trichoderma harzinum*, 1,3-glucanase from barley (*Hordeum vulgare* L.), poly-galacturonase-inhibiting-proteins (pgip) from kiwi (*Actinidia arguta* L.) and raspberry (*Rubus aliceae* L.) and stilbene synthase from grapevine (*Vitis vinifera* L.). For the transformation of mature embryos we used the disarmed hypervirulent *A. tumefaciens* strain EHA 101, furnished with the Bin 19 binary vector, harbouring the combined GOI / bar T-DNA. For the molecular characterisation of 21 independent lines we performed Southern Blot assays in order to confirm their independence. Incomplete integration of T-DNA and gene silencing phenomena were studied as well as the integration of vector backbone sequences. The quantification of this undesired events and the examination of possible impacts of backbone sequences on the expression stability (De Buck et al., 2000) is also a part of our recent work. For the further functional analysis before and after crossing activities, we focussed on single copy of the whole project we also implement a risk assessment for the possible negative impact on the arbuscular mycorrhiza / plant interaction.

P-1019

Enhanced Resistance to *Venturia inaequalis* in Transgenic Apple by a Gene Coding for Hordothionin. J. Janse, J. G. Schaart, K. J. Puite, D. E. A. Florack, R. Groenwold, K. Pelgrom, and F. A. KRENS. BU Genetics & Breeding, Plant Research International, P.O. Box 16, 6700 AA Wageningen, The Netherlands. E-mail: F.A.Krens@plant.wag-ur.nl

The apple cultivars Elstar, Golden Delicious and Gala were used for *Agrobacterium tumefaciens*-mediated leaf-disc transformation using the strain AGL0 containing the plasmid pMOG402.*hth.gus*intron. The *hth* gene is a semi-synthetic gene coding for a type 1 a -hordothionin driven by the CaMV35S promoter. In bioassays hordothionin was able to inhibit growth of *Venturia inaequalis* (apple scab). After transformation, GUS positive shoots were subjected to PCR analysis for confirmation of the transgenic nature. Eventually, 2 Elstar, 2 Golden Delicious and 32 Gala transgenic clones were established, rooted and transferred to the greenhouse. The young plants were subjected to a preliminary scab test and on the basis of these results 2 Elstar, 2 Golden Delicious and 16 Gala clones were propagated on rootstocks for elaborated testing on scab resistance. Both Elstar clones and 4 Gala *hth* clones showed a highly significant reduced susceptibility to scab, compared to the control clones. In the best *hth* clone the reduction in symptoms was almost 50%. Although plant size varied between clones, there was not a clear correlation with scab incidence, indicating that the activity of the *hth* gene was the main cause for this effect. RT-PCR and Northern blot analysis confirmed the expression of the *hth* gene in these clones.

P-1020

Transgenic *Brassica oleracea* var. *alboglabra* with Insect Resistance. K. CHENGALRAYAN[1,2], B. Huang[1], and K.-W. Yeh[1]. [1]Department of Botany, National Taiwan University, Taipei, Taiwan. [2]Present address: Agronomy Department, University of Florida, Gainesville, FL 32611–0300. Email: chengal@ufl.edu

Brassica oleracea var. *alboglabra* (Chinese kale) is a leafy vegetable, commonly grown in Southeast Asia. In Taiwan, at least 3 cultivars of Chinese kale were grown throughout the year. However, this crop is susceptible to several local insect pests including *Spodoptera litura* and *Plutella xylostella*. To combat insect pests, a sweet potato (*Ipomoea batatas* cv. *Tainong* 57) trypsin inhibitor (SPTI) gene was successfully introduced into Chinese kale (*Brassica oleracea* cv. *alboglabra*) via *Agrobacterium*-mediated transformation. Thirty seven percent of transgenic plants were obtained from 5-day pre-cultured hypocotyl explants infected with *Agrobacterium* strain, GV3101, which was grown in AB minimal medium supplemented with 50 mM acetosyringone and co-cultured for 2 days in callus inducing medium containing 10 mg/l $AgNO_3$. In the similar conditions, the frequency of response in LBA4404-infected hypocotyl explants was 31.13%. From 500 independent transformants, 7 lines with high levels of expression were further analyzed. Insecticidal bioassay of transgenic line 68 showed that the larval growth of *Spodoptera litura* was severely retarded as compared to their growth on control plants.

P-1021

Expression of the *Bt* (cry2Aa2) Gene in Transgenic Cotton Chloroplasts. SHASHI KUMAR and Henry Daniell. Department of Molecular Biology and Microbiology, University of Central Florida, 12722 Research Parkway, Orlando, FL 32826–3227. E-mail: daniell@mail.ucf.edu

Cotton is grown in more than 80 countries around the world. Different species of insect pests attack cotton, causes yield loss, and some, such as the cotton bollworm (*Helicovera zea*), totally destroys this crop. So far transgenic cotton cultivars released for commercial use (containing *Bt* gene) have not given any protection against bollworm. Moderate doses of *Bt* tend to accelerate rather than retard the development of pest resistance. Recently, in our laboratory, it has been reported that expression of the *Bt* (cry2Aa2) operon in transgenic chloroplasts of tobacco resulted in the accumulation of the Cry2Aa2 protein at 46.1% of the total soluble protein. Insects that are normally difficult to control (10-day-old cotton bollworm, beet armyworm) were killed 100% after consuming transgenic leaves. Transgenic chloroplast plants pose no environmental risks due to lack of out-cross and offer biological containment as chloroplast genes are maternally inherited. We attempt here to express this gene in cotton chloroplasts. Chloroplast transformation will be accomplished through particle bombardment of embryogenic calli derived from hypocotyl explants of *Gossypium hirsutum* CV. Coker 310. Bt gene driven by the psbA promoter and the BADH gene (from spinach) will be used for selection of transgenic tissues, since embryogenic tissues of cotton are very sensitive to antibiotic selection. The selection process involves conversion of toxic betaine aldehyde to glycine to glycine betaine by BADH. Transgenic cotton plants will be confirmed by PCR, southern blot analysis, ELISA and insect bioassays will be performed.

P-1022

Virus Resistance in Transgenic Fruit Trees: The Technical Feasibility, the Environmental Impact, the Public Acceptance. MARGIT LAIMER. IAM-BOKU, Nussdorfer Lande 11, Vienna, AUSTRIA, A-1900. Email: m.laimer@iam.boku.ac.at

Breeding of woody plants requires long time periods due to the long generation time, the high degree of heterozygozity and to need for multiple backcrosses to eliminate undesired traits. In many instances resistance genes against biotic and abiotic factors are not even available in closely related species of cultivated fruit crops, but occur in wild species or non cultivated cultivars, which have only a poor fruit quality. The application of molecular techniques makes new resistance genes available and breeding steps will take less time, if desired traits can be directly introduced into high yielding cultivars. This need concerns not only new cultivars important for the actual market, but also the conservation of old local cultivars, which are endangered by the spread of certain pathogens. We have focused our efforts on virus resistance, since there does not exist any chance to control these pathogens by chemical means, and the chemical control of their vector organisms, e.g., aphids, nematodes, appears ecologically highly questionable. Agrobacterium tumefaciens transformation was chosen as method, since fruit belong to the natural host range of these pathogens. Following the pathogen-mediated protection approach, we have isolated the coat protein gene of the stone fruit pathogens PPV (Laimer da Câmara Machado et al., 1992. Plant Cell Reports 11(1): 25–29) PNRSV (Hammond et al., unpublished) and of four grapevine viruses (Gölles et al., 1998. Acta Hort. 528:305–311) and transformed different explants of different woody species. It is obvious that the main obstacle for transformation of fruit tree species is the regeneration of transformed plantlets. Attempts to improve crop plants by genetic engineering techniques will therefore depend very strongly on the availability of reliable protocols for transformation, selection and regeneration. Furthermore regeneration of plants from single cells or complex explants is a precondition for Agrobacterium tumefaciens mediated gene transfer to achieve homogeneously transformed plants. To this purpose different explants with their different developmental patterns are suited in a different manner. The use of leaf discs from some species (apple, plum, grapevine) was compared to somatic embryogenesis (cherry, grapevine), which definitively offers the advantage of single cell regeneration and therefore currently appears to be the most promising approach to introduce new genes in woody crop species (da Câmara Machado et al., 1995. Plant Cell Reports 14:335–340). The integration of the various viral coat-protein genes as well as of some marker genes has been demonstrated by PCR and by Southern Blot analyses (Gölles et al., 1998). The majority of transgenic plants analyzed so far contained only one or a few copies of the transgene, which in terms of further evaluation and segregation seems advantageous. With the aim to gain public confidence in and acceptance for these techniques we have engaged in a programme to bring these plants stepwise into an insect proof screenhouse and into field trials (http://www.boku.ac.at/sicherheitsforschung).

P-1023

High Throughput Identification of Insect Resistance Genes. SUSAN D. LAWRENCE, Nicole G. Novak, and Jeffrey M. Slack. USDA-ARS, Insect Biocontrol Laboratory Bldg. 011A, Rm. 214, Beltsville, MD 20705. E-mail: lawrencs@ba.ars.usda.gov

Rapid identification of genes that confer resistance to insect pests would greatly augment molecular breeding programs. The initial analysis of recombinant genes in transgenic plants usually occurs after months of labor intensive nurturing of tissue culture grown material. A vector initially designed by Baulcombe et al. expresses a gene in young seedlings within weeks via potato virus X (PVX). We have modified this vector allowing ectopic expression of a recombinant protein with a short epitope tag. Not only does the protein remain functional, but also any recombinant protein can be specifically identified from endogenous plant proteins. We have tested four putative resistance genes using an assay measuring larval development or mortality of either Colorado potato beetle or tobacco budworm. Two of the genes are a chitinase and the proteinase, V-CATH both from the insect baculovirus AcMNPV. The other two are *win-4* and *win-6*, systemically wound-induced genes from poplar. This type of approach should drastically shorten the time required to select promising genes that deter such lepidopteran and coleopteran pests.

P-1024

The Maize Hm1 Gene as a Selectable Marker for Maize Transformation. MICHAEL MILLER, Gary Sandahl, Bob Meeley, Susan Grant, Diane Bond-Nutter, and Bill Gordon-Kamm. Pioneer Hi-Bred International, Inc., Johnston, IA 50131. E-mail:michael.miller@pioneer

HC-toxin isolated from Cochliobolus carbonum race 1, an inhibitor of maize histone deacetylase, was used to select HiII maize transformed with the maize Hm1 gene. The maize Hm1 gene encodes for NADPH-dependent HC-toxin reductase, which inactivates HC-toxin, a cyclic tetrapeptide. HiII immature embryos were used as particle bombardment targets where vigorous, clearly identifiable callus transformants developed eight to twelve weeks later using 1 mg/L HC-toxin for selection. A direct comparison between HC-toxin and bialaphos selection was performed where embryos co-bombarded with PAT and Hm1 were split equally between bialaphos and HC-toxin selection. The average transformation frequency, in terms of number of bombarded embryos producing transgenic events, for HC-toxin selection was about half that of bialaphos selection (1.9% and 3.9% respectively). Healthy, viable plants were readily regenerated from Hm1 transformed callus. An assay was developed where Hm1 transformed T_0 HiII plants are distinguished from non-transformed plants by painting 1.5 mg/ml HC-toxin in lanolin onto leaves. In bioassays on segregating Hm1 positive T_1 seedlings, the presence of the Hm1 expression cassette correlated with increased fungal (Cochliobolus carbonum) resistance.

P-1025

Transformation of a Commercial Barley Cultivar with Genes for Resistance to Fusarium Head Blight. M. MANOHARAN[1], L. S. Dahleen[2], T. Hohn[3], S. P. McCormick[4], N. A. Alexander[4], P.Schwarz[5], and R. D. Horsley[1]. [1]Department of Plant Science, North Dakota State University, Fargo, ND 58105; [2]USDA-ARS Northern Crop Science Laboratory, Fargo, ND 58105; [3]Syngenta, Inc., Research Triangle Park, NC 27709; [4]USDA-ARS, National Center for Agricultural Utilization Research Laboratory, Peoria, IL 61604; and [5]Department of Cereal Science, North Dakota State University, Fargo, ND 58105. E-mail: dahleenl@fargo.ars.usda.gov

Fusarium head blight, incited primarily by *Fusarium graminearum*, has caused devastating losses to barley since the 1990's. Production of the mycotoxin deoxynivalenol (DON) by *F. graminearum* is harmful to humans and livestock. Expressing certain anti-toxin genes such as *TRI*101 and *PDR*5 could improve resistance to fungal infection and reduce DON levels. *TRI*101 encodes a 3-OH trichothecene acetyltransferase that converts DON to a less toxic acetylated form. *PDR*5, an ATP-binding cassette, acts as an efflux transporter, shunting DON across the plasma membrane from the interior of the cell. We have transformed the commercial malting barley cultivar Conlon with these genes to reduce DON levels in infected grain. Ten-day old calli derived from immature embryos were co-bombarded with the herbicide-resistance gene bar as the selectable marker. Putative transgenic plants were confirmed by Southern analysis. A total of seven independent events with *TRI*101 and six with *PDR*5 were recovered. Northern analysis indicated the expression of *PDR*5. Expression of *TRI*101 was confirmed by detecting acetyltransferase activity in seeds of the transgenic plants. T^2 lines of three events with *TRI*101 and two events with *PDR*5 were field tested for disease and toxin level. Both genes appeared to reduce FHB infection and *PDR*5 also may reduce DON accumulation.

P-1026

Biotechnology for Oat Improvement. ANNA MARIA NUUTILA, Elina Kiviharju, Kirsi Lehto, Eero Nissilä, and Kirsi-Marja Oksman-Caldentey. VTT Biotechnology, FIN-02044 VTT, Espoo, Finland; MTT, Plant Production Research, Crops and Biotechnology, Jokioinen, Finland; University of Turku, Department of Plant Physiology and Molecular Biology, Turku, Finland; Boreal Plant Breeding Ltd., Jokioinen, Finland. E-mail: anna-maria.nuutila@vtt.fi

In Finland the annual oat (*Avena sativa* L.) production ranks second only behind barley. Finland is one of the major oat producers in the world and the Finnish share of the worldwide oat trade is approximated to be 20%. In recent years reports of the beneficial nutritional and physiological effects of oat products have increased interest in oats as an important food source. In order to improve the Finnish oat cultivars better to meet the requirements of the food industry, modern biotechnical methods have been used. Cell cultures have been started from mature embryos of six Finnish oat cultivars, from two cultivars also leaf bases were used. Callus and green plantlets were obtained from all of these cultivars. Additionally methods for double haploid production through anther culture have been developed. Gene transfer has been accomplished using particle bombardment. Our first aim was to develop oat varieties with resistance to BYDV (barley yellow dwarf virus) through gene transfer. The T_1 progeny of the transgenic plants has been tested for viral resistance against BYDV. The T_2 and T_3 progeny are presently being tested.

P-1027

Biolistic Transformation, Recovery, and Expression of Fertile Soybean Transgenics for Chitinase and *Pto* Genes. WOJCIECH ORNATOWSKI[1], William T. Schapaugh[1], Subburatnam Muthukrishnan[2], and Harold N. Trick[3]; [1]Department of Agronomy, [2]Department of Biochemistry, [3]Department of Plant Pathology, Kansas State University, Manhattan, KS 66506. E-mail: htrick@ksu.edu

Our long-term goal is to control soybean diseases through the use of genetic transformation. The diseases we have specifically targeted include charcoal rot, caused by the fungal pathogen *Macrophomina phaseolina* and Soybean Cyst Nematode (SCN) caused by the nematode *Heterodera glycines*. One of our approaches is to constitutively overexpress plant and insect chitinase genes. Our hypothesis is that overexpression these proteins will reduce the virulence or fitness of the fugal pathogen or SCN. Alternatively, we also have overexpressed the tomato gene (*Pto*) conferring resistance to *Pseudomonas syringae*. Overexpression of the *Pto* gene in tobacco has been demonstrated to confer broad resistance to bacterial, fungal, and viral pathogens. The experimental strategy was to biolistically transform soybean with a vector DNA containing a rice chitinase gene (*chi11*), tobacco hornworm chitinase gene (*msc*), and *Pto* gene, driven by the CaMV 35S promoter and linked to the *hpt* gene as a selectable marker. Immature embryos of soybean cultivars 'Chapman', 'Jack', and 'Fayette' were bombarded, and several independent clones were selected on hygromycin-containing media and regenerated into plants. The majority of transgenic plants were morphologically normal and self-fertile. The integration, inheritance and expression of the genes have been confirmed by molecular analysis of T_1 and T_2 soybean transgenic plants. Independent transformants have been confirmed by the polymerase chain reaction (PCR) to contain our selectable (*hpt*) marker gene and the gene of interest. The presence and estimated copy number of inserts were detected by Southern blot analysis. Northern blotting and Western blotting confirmed the expression of transgenes. Progeny from the *Pto* and chitinase-positive plants were tested for their resistance to the charcoal rot and soybean cyst nematode. The degree of resistance displayed by these transgenic plants was correlated with the level of the genes expression.

P-1028

Analysis of Transgenic Sugarcane Plants Containing an Untranslatable Sugarcane Mosaic Virus Strain E Coat Protein Gene. M. OROPEZA[1], A. M. Abouzid[1], J. D. Miller[2], J. C. Comstock[2], R. A. Gilbert[3], and M. Gallo-Meagher[1]. [1]University of Florida, Agronomy Department, Gainesville, FL 32611–0300, [2]USDA-ARS, Canal Point, FL, and [3]University of Florida, Everglades Research and Education Center, Belle Glade, FL. Email: moropeza@ufl.edu

Sugarcane mosaic virus strain E (SCMV-E), the most prevalent strain in Florida, recently has been observed in much greater frequency and extent than ever before in the Everglades Agricultural Area. To evaluate a transgenic approach for obtaining resistance to SCMV-E, an untranslatable SCMV-E coat protein gene under the control of the maize *Ubi-1* promoter was co-bombarded along with the *nptII* gene into callus produced from two sugarcane varieties, CP 80–1827 and CP 84–1198. Approximately, 190 transgenics were initially screened for SCMV-E resistance in the greenhouse, and 106 of these were placed in replicated field trials. Field screening allowed for selection of 40 transgenics that possessed desirable agronomic characteristics, and resistance to natural SCMV-E infection via aphid transmission. Molecular characterization and continued field evaluation for SCMV-E resistance of these plants are leading to an understanding of the mechanism and stability of SCMV-E resistance.

P-1029

Improving Resistance to *Alternaria triticina* in Wheat Plants Transformed with a Barley Gene Encoding a Family 5 Pathogenesis-related Thaumatin-like Protein. M. M. Salgado[1], R. I. W. Osmond[2], A. PELLEGRINESCHI[1], R. Reid[2], M. Mezzalama[1], D. Hoisington[1], S. Donner[2], R. A. Burton[2], and G. B. Fincher[2]. [1]Applied Biotechnology Center, CIMMYT, Apdo Postal 6–641, 06600 Mexico, D.F.; [2]Department of Plant Science, University of Adelaide, Waite Campus, Glen Osmond, SA 5064, Australia; and [3]CIMMYT Wheat Program, CIMMYT, Apdo Postal 6–641, 06600 Mexico, D.F. E-mail: M.Salgado@cgiar.org

The use of genetic engineering for the incorporation of resistance in susceptible plants is an alternative strategy to classical breeding for developing crop species with resistance to fungal pathogens. Thaumatins are potentially useful candidate genes that code for pathogenesis-related (PR) proteins with antifungal properties. Enhanced PR proteins levels in transgenic plants reduce or eliminate the damages caused by pathogens as *Alternaria triticina*. Transgenic wheat plants were obtained by co-transformation with PR-5 gene constructs and the *bar* gene, using particle bombardment of Bobwhite and Baviacora (CIMMYT elite wheat cultivar). The plasmid constructs used were pCAMBIA1390 containing the corresponding thaumatin gene (sense or antisense) under the control of the ubiquitin promoter and the plasmid pAHC25 containing *bar* as selectable marker gene, also under the control of the ubiquitin promoter.The transfomed plants were selected by their resistance to the herbicide Basta. Molecular analysis of isolated DNA by PCR indicated the presence of bar gene in 407 Basta-resistant plants, but only 113 were positive to the *Htp* gene of the pCAMBIA 1390 plasmid. Detection by PCR of the barley transgene (PR-5), without interference by the endogenous wheat thaumatin-like protein gene, was possible after specific primers were developed through the analysis of ESTs of endogenous wheat thaumatin.The transgenic plants were evaluated and selected by their tolerance or resistance to infection by *Alternaria triticina*, under controlled conditions. The in vitro and in vivo bioassays indicated different levels of resistance in transgenic plants to pathogen attack. The progeny of the event no. 2142 improved their resistance to *A. triticina*. Western Blot Analysis using polyclonal antibodies showed different patterns of expression of thaumatin-like proteins in transgenic plants that seemed to be related to fungal resistance to *A. triticina*. However, this analysis, using polyclonal antibodies, was not ideal for detecting the expression of the thaumatin transgene because of the presence of endogenous thaumatin-like proteins. The transcriptional expression of thaumatin has been detected by Northern hybridization analysis in two plants (T1 generation) of the primary transformant no. 2142. This plant showed resistance to *Alternaria triticina* and the presence of the thaumatin transgene was confirmed by PCR and Southern blot analysis in T1 and T2, and T3 plants. Several copies (at least 5) were detected by Southern blot analysis in most of these plants. The plants derived from transformation line no. 2142 improved significantly their resistance to *A. triticina*.

P-1030

Tissue Culture of American Ginseng (*Panax quinquefolius* L.) and Genetic Engineering to Express Antifungal Proteins through *Agrobacterium* Transformation. ZAMIR K. PUNJA, W. P. Chen and M. Feeney. Centre for Environmental Biology, Department of Biological Sciences, Simon Fraser University, 8888 University Drive, Burnaby, British Columbia V5A 1S6. E-mail: punja@sfu.ca

American ginseng (*Panax quinquefolius* L.) is a slow-growing herbaceous perennial plant that is cultivated for its highly valued root, which provides a source of vitalizing and stimulating agents. The establishment and potential yield of ginseng plants can be reduced by seed-borne diseases and root and stem-infecting fungi. To potentially enhance tolerance of American ginseng to fungal diseases, we introduced a rice chitinase gene and a thaumatin-like protein gene using *Agrobacterium*-mediated transformation. The genes were under control of the maize ubiquitin 1 promoter. The phosphinothricin acetyltransferase *(bar)* and hygromycin phosphotransferase *(hpt)* genes were used as selectable markers. For transformation, seedlings epicotyl explants were each infected with 10–15 µl droplets of *A. tumefaciens* strain LBA 4404, cocultured for 3–4 days, and selected using 20 mg/L phosphinothricin or 100 mg/L hygromycin. The tissue culture medium was MS with 10 µM NAA and 9.0 µM 2,4-D. A callusing frequency of 24–27% was achieved on selection medium after 10 mo in culture, and 90% of the lines were shown to be transformed by PCR and Southern analyses. The expression of the chitinase and TLP genes was confirmed by RT-PCR and Western analyses. Transformed calli were propagated in suspension cultures and developing somatic embryos were plated onto MS medium with 1% activated charcoal. Root and shoot growth was enhanced by a 7-day exposure to 3 µM GA_3 and 5 µM BA. Plantlets were recovered from somatic embryos of 11 confirmed transgenic lines and are being acclimatized prior to disease tests.

P-1031

Almond (*Prunus dulcis* MILL.): Preparing and Testing of Constructs for Resistance to Prune Dwarf Virus (PDV). H. RAQUEL[1,2], T. Lourenço[1,2], R. Batista[1,3], M. M. Oliveira[1,2]. [1]ITQB/IBET, Quinta do Marquês, 2780, Oeiras, Portugal; [2]Dep. Biologia Vegetal, Fac. Ciências Lisboa, Campo Grande, 1780 Lisboa, Portugal; and [3]Inst. Ricardo Jorge, Av. Padre Cruz, 1699 Lisboa, Portugal. E-mail: hraquel@itqb.unl.pt

Prune dwarf (PDV) and *Prunus necrotic ringspot* (PNRSV) viruses are responsible, in Portugal as well as in other countries, for the productivity losses in almond orchards. As these viruses can be transferred by pollen and almond is cross pollinated, it is very difficult to maintain clean (virus-free) orchards. Genetic engineering using the coat protein (CP) strategy has been the most successful method to achieve plant resistance to virus infection. Aiming to introduce resistance to *Prune dwarf virus* (PDV) in Portuguese almond genotypes, we have been preparing and testing constructs carrying the PDV coat protein gene. Using the IC/RT-PCR strategy, the CP gene of PDV was isolated from infected almond tissues and cloned in intermediate vectors. The CP gene sequences, in sense or antisense orientation, were cloned in pGreen (John Innes Center) transformation vectors. To reduce putative repression influences exerted by flanking plant DNA, or undesirable interactions of multicopies of the transgenes, matrix attachment regions (MARs from tobacco, Rb7) (J. Allen, NC State University) have been inserted flanking the cloned CP chimeric genes. All the constructs were amplified in *E. coli* and then transferred to *Agrobacterium* EHA105. Prior to use in almond transformation, these constructs are being tested in the transformation of *Nicotiana* species. Transgenic *Nicotiana* plants expressing the cpPDV were already regenerated and are being tested for virus resistance. Acknowledgements- Praxis 3/3.2/HORT/2143/95, Fellowship BD 19631/99 of HR.

P-1032

Genetic Engineering of Grapevine Rootstocks to Induce Nepovirus Resistance. G. M. REUSTLE, T. Wetzel, R. Jardak*, R. Ebel, R. Wolf, L. Meunier, M. Becker, G. Krczal. Centrum Gruene Gentechnik, SLFA Neustadt, D-67435 Neustadt/W. *INRST Biotechnologie Végétale, B.P. 95, 2050 Hammam-Lif, Tunisia. E-mail: greustle.slfa-nw@agrarinfo.rlp.de

The major virus disease in German viticulture is the Fanleaf disease, caused by a group of nepoviruses, the Grapevine Fanleaf Virus (GFLV), Arabis Mosaic Virus (ArMV) and Raspberry Ringspot Virus (RRV). The viral infections dramatically decrease the value of an infested vineyard because of the decrease in both the yield and quality of the grapes. Due to a lack of natural genetic resources for virus resistance, suitable for cross breeding programs, a transgenic approach was chosen to develop rootstocks resistant against these nepoviruses. As the viruses are transmitted by nematodes only, resistance of rootstocks against these viruses would be sufficient to prevent infection of the grapevines. To establish constructs possibly inducing gene silencing, highly conserved sequences (movement protein of GFLV and ArMV, 5' non coding region of RNA-2 of RRV) from the viruses were combined with defective interfering (DI)-sequences from Potyvirus and/or used to clone inverted-repeat constructs. Evaluation of the constructs in tobacco (*N. benthamiana*) yielded lines showing immunity, retarded infection and non resistance. Further analysis to confirm gene silencing mechanism in the tobacco lines are in progress. For genetic engineering of grapevine, embryogenic tissue of rootstocks (SO-4, 125-AA, 5C, Binova) was induced from anther cultures and used for Agrobacterium mediated transformation. After 16 to 20 weeks of selection on phosphinothricin (PPT) containing media (1.0; 2.5; 5.0 mg/L), new generated somatic embryos were harvested form the original explants and cultivated on PPT free media for regeneration. PCR analysis of embryos demonstrated a high level of escapes. Depending on the selection procedure (concentration, duration, interval of media changes) 5 to 30% of the regenerated somatic embryos were transgene positive. Transgenic grapevines could be regenerated from these embryos.

P-1033

Studies of Plum Pox Virus Resistance in Transgenic Plum C5 and Its Progeny. RALPH SCORZA[1], Michel Ravelonandro[2], Ann M. Callahan[1], Tadeusz Malinowski[3], Vern D. Damsteegt[4], Laurene Levy[5], and Pascal Briard[2]. [1]USDA-ARS-Appalachian Fruit Research Station, Kearneysville, WV; [2]UMR GDPP-Virologie, INRA, Bordeaux, France; [3]Research Institute of Pomology and Floriculture, Skierniewice, Poland; [4]USDA-ARS FDWSR, Fort Detrick, MD; and [5]USDA-APHIS CPHST-NPGQC, Beltsville, MD. E-mail rscorza@afrs.ars.usda.gov

Plum pox virus (PPV) is one of the most devastating diseases of *Prunus* (stone fruit) species. Since the first report of PPV in Bulgaria in the early 1900's PPV has spread throughout Europe, into parts of Asia and Northern Africa, and more recently to Chile, the U.S. and Canada, where eradication efforts are currently underway. Breeding for resistance is an important control strategy but few sources of high level resistance have been identified, and these are multigenic, requiring long-term breeding efforts for their incorporation into commercial cultivars. Transgene-based resistance offers a complementary approach to developing PPV-resistant stone fruit cultivars. Transgenic plum clone C5 carrying the PPV coat protein (CP) gene was found to be highly resistant to PPV in greenhouse graft-inoculation tests. C5 also displayed high levels of resistance in field tests in Poland, Romania, and Spain. These field tests have suggested that C5 is immune to PPV by aphid inoculation. C5 has been shown to be resistant to all of the major PPV strains tested. Resistance is mediated through post-transcriptional gene silencing. The transgene, which appears to be a complex multicopy repeat and rearrangement of the original gene cassette, is inherited in crosses to *Prunus domestica* and *P. spinosa* as a single dominant locus with all resistant progeny carrying the PPV insert. Our work with clone C5 and its progeny indicates the usefulness of transgene-mediated resistance to PPV, the efficiency of gene-silencing in a woody perennial tree crop, and provides new and useful transgenic germplasm for developing new PPV-resistant lines through hybridization.

P-1034

In Vitro Screening of Sugarcane (*Saccharum* spp) Varieties to Evaluate Smut (*Ustilago scitaminea* Sydow) Susceptibility. N. SINGH, B. M. Somai, and D. Pillay. School of Life & Environmental Sciences, University of Durban-Westville, Private Bag X54001, Durban 4000, South Africa. E-mail:nsingh@pixie.udw.ac.za

In pursuit of a suitable, rapid smut-screening method to eliminate sugarcane genotypes rated as susceptible and highly susceptible, tissue cultured plantlets of 3 sugarcane varieties of known smut reaction were screened. *In vitro* inoculation of these plantlets with + and − sporidia of *U. scitaminea* in equal proportions at a concentration of 1 x 10^8, yielded whip production in NCo310 (highly susceptible), and N12 (resistant-intermediate), but not in N19 (resistant), which correlates with each variety's known smut field reaction. Whips were produced between 60 and 90 days following inoculation at 26° ± 2°C. They were not produced in plantlets inoculated with sporidia of only one mating-type. Traditional methods of screening sugarcane varieties for smut disease usually take 6 to 18 months to complete and requires large areas of cane-land. Furthermore, field trials are variable because of seasonal differences in weather, whereas a tissue culture test under controlled conditions would provide more consistent results. PCR assay for the detection of smut in inoculated plantlets using *U. scitaminea b*E primers, was able to detect *U. scitaminea* DNA in all smut-inoculated plantlets and, was positive for smut strains from different geographic regions of the world. The PCR assay was negative in control plantlets inoculated with sterile distilled water. The results indicate that this *in vitro* smut-screening technique may provide an alternative to lengthy field-testing.

P-1035

In Vitro and Genetic Transformation Studies in Pigeonpea [*Cajanus cajan* (L.)Millsp.]. S. MANOJ KUMAR, K. K. Sharma*, and Prathiba Devi. Biotechnology Laboratory, Department of Botany, Osmania University, Hyderabad 500 007. *ICRISAT, Patencheru, Near Hyderabad. Email: manoj_sriram@hotmail.com

As part of the project on the [*Cajanus cajan* (L.)Millsp.] production of disease resistant transgenic pigeonpea*in vitro* culture conditions for plantlet regeneration from various pigeonpea explants (cotyledonary node, axillary bud and leaf) have been optimized. Efficient production of healthy multiple shoots from the cotyledonary node explants was achieved when cultured on MS medium supplemented with different concentrations of phytohormones. The shoots could be sufficiently elongated with an additional supplementation of phytohormones, successfully rooted on basal MS medium and transplanted to pots with 90% survival. Plantlet regeneration was also achieved with a high frequency from the leaf explant, followed by efficient rooting, transplantation and 95% survival. Optimal parameters for the genetic transformation of the pigeonpea explants by particle bombardment as well as the *Agrobacterium* mediated method have been standardized with GUS gene construct followed by histochemical analysis. With a view to obtain disease resistant pigeonpea transgenics, we are presently carrying out the transfer of rice chitinase gene.

P-1036

In Vitro and Genetic Transformation Studies in Sorghum. D. SYAMALA and Prathiba Devi. Department of Botany, Osmania University, Hyderabad 500 007, India. E-mail: syamala_kumar@yahoo.com

In Vitro culture conditions for plantlet regeneration from sorghum shoot-tip explants have been optimized. Efficient production of healthy multiple shoots directly from shoot tip explants of sorghum has been achieved apart from plantlet regeneration from embryogenic callus when cultured on MS medium supplemented with different concentrations of phytohormones. The shoots could be sufficiently elongated with an additional supplementation pf phytohormones, successfully rooted on basal MS medium and transplantede to pots with 85% survival. Optimal parameters for the genetic transformation of sorghum explants by particle bombardment have been standardized by using the GUS gene constuct followed by the histochemical assay. Molecular analysis of putatively transgenic sorghum plantlets by the PCR method has been carried out. With a view to obtain disease resistant sorghum transgenics, a rice chitinase gene has been transferred and recovery of putatively transformed plants is in progress.

P-1037

Spatial and Temporal Expression of the Sweet Protein (Thaumatin II) Gene in Transgenic Cucumber Plants. MARIA SZWACKA, Ewa Piworun, Agnieszka Ozdoba, Tomasz Szeląg, and Ewa Urbańczyk-Wochniak. Department of Plant Genetics, Breeding and Biotechnology, Warsaw Agricultural University, Nowoursynowska 166, 02–787 Warsaw, Poland. Email: szwacka@alpha.sggw.waw.pl

Thaumatin (22 kD) is an extremely sweet-tasting basic protein synthesized in the fruits of the West African shrub *Thaumatococcus daniellii* Benth. By the immunoblot analysis, it was initially shown that thaumatin protein levels varied spatially and temporally in T_3 progeny of four independent transgenic cucumber plants (*Cucumis sativus* L.) harboring the 35S-thaumatin II chimaeric gene. The highest amount of transgenic protein was produced in the roots. The spatial distribution of the thaumatin protein was determined immunocytochemically in leaf tissues and floral organs of T_3 and was consistent with distribution of thaumatin mRNA determined by *in situ* RT-PCR analysis. The major locations of thaumatin mRNA and protein were in the epidermal layer of the leaves and in some tissues of male (vascular bundle, receptacle and perianth) and female (perianth and style) flower buds. Previously, it was shown that some of the T_2 plants expressing the thaumatin gene exhibited different levels of the protection against *Pseudoperonospora cubensis*. Thaumatin belongs to the pathogenesis-related (PR) protein family. Its presence in the epidermis of transgenic cucumber plants could play a role in enhancing the resistance of these plants to pathogens. The results allow to conclude that the 35S promoter is not constitutive or that there are some mechanisms that regulate the level of the transgene expression products. This work was supported by grant No. 5 PO6A 02518 from State Committee for Scientific Research (M. Sz.).

P-1038

Expression of Rp1-D Resistance Genes in Transgenic Maize and Wheat. Martin Steinau[1], Michael A. Ayliffe[2], Tony A. Pryor[2], Nicholas C. Collins[2], Scot H. Hulbert[1], and HAROLD N. TRICK[1]. [1]Department of Plant Pathology, Kansas State University, Manhattan, KS 66506. [2]Division of Plant Industry, Commonwealth Scientific and Industrial Research Organization, Canberra, ACT 2601, Australia. E-mail: htrick@ksu.edu

The Rp1 complex of maize consists of a family of nucleotide-binding site, leucine-rich repeat genes (NBS-LRR) which control resistance to common leaf rust (*Puccinia sorghi*). The *Rp1-D* gene promotes race-specific rust resistance while the recombinant *Rp1-D21* gene is thought to confer a non-specific reaction to *Puccinia* isolates resulting in a necrotic spotting lesion mimic phenotype. A genomic clone of the *Rp1-D* gene with its native regulatory elements and a construct with the strong ubiquitin promoter were transformed into susceptible maize lines via biolistics. The resulting T_0 plants were backcrossed one or more times to the maize lines HII and H95 to identify stably expressing rust resistance. Different levels of rust resistance were observed in several independent transgenic lines. It is likely that different phenotypes can be associated with expression levels. Two maize lines expressing a copy of the *Rp1-D21* gene were constructed and both exhibited the non-specific necrotic spotting phenotype. Both the *Rp1-D* and the *Rp1-D21* genes were also transformed into wheat to analyze their utility and functionality in evolutionary divergent cereals. 16 independent *Rp1-D* transgenic lines and 24 of *Rp1D–21* were analyzed up to the T_4 generation and approximately 30% of them showed stable transcription of the transgenes. None of these lines however exhibited any noticeable increase in resistance to wheat leaf rust *(Puccinia triticina)* or any chlorotic or necrotic spotting.

P-1039

Genetic Engineering of Wheat and Sorghum with Genes Encoding Pathogenesis- related Proteins (PR) to Enhance Disease Resistance. Ajith Anand[1], Jayaraj Jayaraman[1], Haiqing Yi[1], Saminaidu Krishnaveni[1], Jeoung-Mee Jeoung[2], Juliane S. Essig[3], HAROLD N. TRICK[3], George H. Liang[2], and Subbaratnam Muthukrishnan[1]. [1]Department of Biochemistry, [2] Department of Agronomy[3], Department of Plant Pathology, Kansas State University, Manhattan, KS 66506. E-mail: ajith@ksu.edu

Introducing genes for pathogenesis-related proteins (PR) and the pyramiding of these genes might be a promising strategy to elevate the levels of resistance to diverse pathogens in wheat and sorghum. We have genetically engineered wheat and sorghum with genes for a single and combinations of PR- proteins including chitinase(s), beta1,3-glucanase(s) and thaumatin-like protein (TLP), to identify the best gene or gene combinations for resistance to pathogens. Transformed wheat (Bobwhite) calli from immature embryo were selected on 5 mg/L glufosinate and 24 independent transgenic lines were characterized. Four of these lines which showed stable inheritance and expression of the transgenes in T_3 generation were identified. Preliminary bioassay results with scab will be presented from 2 trials for some of these lines. A homozygous line co-expressing a chitinase/glucanase combination with enhanced resistance to scab was crossed to TLP transgenic wheat (moderate resistance) for gene pyramiding. Transgenic sorghum plants expressing a rice chitinase (Chi11), or a rice thaumatin-like protein (TLP) were obtained through biolistic and Agrobacterium-mediated transformation, using immature embryos as explants and *bar* gene as the selectable marker. Progeny analyses of the T_3 plants confirmed the stable inheritance and expression of the transgenes. The transgenic plants were also screened for resistance to *Fusarium* stalk rot disease, which is a major disease of sorghum. The transgenic plants with *chi*11 chitinase gene showed moderate level of resistance, when compared to the non-transgenic controls, while the transgenic plants expressing the *tlp* gene, showed enhanced level of drought resistance. Results with the cyanamide hydratase gene (*cah*) as a selectable marker in sorghum will be presented.

P-1040

In vitro and *ex vitro* Selection of Resistant Somaclones to *Phytophthora infestans* (Mont.) De Bary in Diacol Capiro Potato (*Solanum tuberosum* L.) Variety. AURA INÉS URREA-TRUJILLO. Instituto de Biología. Universidad de Antioquia. A.A.1226 Medellín, COLOMBIA. Fax: (574)2330120. Email: aurrea@matematicasudea.edu.co

In order to evaluate the possibility to find resistance to *Phytophthora infestans* among a susceptible population of *Solanum tuberosum* var. Diacol Capiro a research work was carried out in three different steps:— first, callus culture were irradiated with different gamma ray doses to increase variability on Diacol Capiro. Second, a highly pytotoxic culture filtrate (CF) of *P. infestans* was obtained to be used as selecting agent at *in vitro* level on irradiated (15 Gy) and non irradiated callus which were exposed to the CF over two cycles of selection. Regenerated plants were used as hosts in designed experiments to select them for *P. infestans* resistance. The third and final step was performed to select resistance traits under greenhouse and field conditions. From this attempt, the dose limitation range (GR50-DL50)for callus of Diacol Capiro was 12–17 Gy; and 15 Gy were selected. 10% of irradiated callus survived after exposure to CF. Callus culture were transfered to a regeneration media and give rise to 221 somaclones. After first cycle of selection (under greenhouse conditions), 39 genotypes were chosen to be evaluate for resistance to the disease and productive potential (Kg/plant). From the irradiated callus, four somaclones shown a significative differences for r-value (relative infection rate)and the productive potential. This work introduces a new scheme of *in vitro* somaclones potato selection in order to be used in breeding programs to *Phytophthora infestans* resistance.

P-1041

Resistance to Rice Yellow Mottle Virus Disease in African Rice. B. R. WOODWARD, J. W. Snape, and M. L. Koyama. Crop Genetics Department, John Innes Centre, Norwich Research Park, Norwich, NR4 7UH, United Kingdom. E-mail: barbara.woodward@bbsrc.ac.uk

Rice yellow mottle virus (RYMV) causes a serious endemic disease in irrigated and rainfed lowland rice cultivation in Africa and Madagascar. Since the introduction of susceptible Asian varieties in the 1960s, this highly infectious disease has reached epidemic proportions. Some success has been achieved using conventional breeding techniques at the West African Rice Development Association (WARDA), to produce resistant interspecific varieties. In order to pyramid resistance, a collaborative, needs-driven strategy, funded by the Department for International Development was devised to produce transgenic resistance to RYMV in African rice cultivars. This approach is based on a pathogen-derived resistance mechanism, where a highly conserved gene, or part of a gene, derived from a pathogen is transgenically expressed in the host, thereby halting the infection process. Previously, this strategy was shown to be highly effective. More recently, alternative regeneration techniques for recalcitrant cultivars using mature embryos have been developed. New constructs have also been produced to replace the antibiotic hygromycin selection gene with a phosphomannose isomerase (PMI) gene for positive selection. These constructs, which contain part of the RYMV genome, are being used to transform rice using particle bombardment, in order to confer RNA-mediated resistance by activation of posttranscriptional gene silencing. This study shows how biotechnology may be combined with conventional breeding approaches to implement an effective, more robust, strategy for combating this serious disease.

P-1042

Tomato Plants Expressing Bcl-xl and Ced-9 Genes Show Altered Necrosis Symptoms after Infection by Cucumber Mosaic Virus/D Satellite RNA. P. XU, S. J. Roger, and M. J. Roossinck. The S. R. Noble Foundation, P. O. Box 2180, Ardmore, OK 73402. E-mail: pxu@noble.org

Cucumber mosaic virus (CMV) and D satellite RNA (satRNA) cause a lethal disease in tomato and no natural resistance has ever been found. Previously we showed the involvement of programmed cell death (PCD) in the disease development. Very little is known about the regulation and execution of plant PCD, but several pathways in animal and nematode PCD are well-studied. Here, tomato plants expressing animal and nematode PCD negative regulators Bcl-xl and Ced-9, were generated through agrobacterium transformation. The seeds were harvested and the F1 seedlings positively expressing the transgenes were inoculated with CMV and D satRNA. About 18%-33% of the inoculated seedlings from five transgenic lines survived the inoculation test with either conspicuously delayed cell death or without symptoms, and produced fruit. The necrotic tissue from the plants with delayed cell death and the systemic leaves from surviving plants were harvested and the total RNA was extracted. Results from reverse transcription and PCR for CMV RNA3 and D satRNA suggest the successful infection of both CMV and D satRNA in the plants. However, only CMV RNA3 was detected in the systemic leaves of all the plants from one transgenic line and a few plants from other lines. Therefore, the expression of Bcl-xl or Ced-9 in tomato plants may participate in negative regulation of CMV and D satRNA induced PCD and may also affect the accumulation of D satRNA.

P-1043

Characterization of Viruses Infecting Transgenic Papaya Resistant to Papaya Ringspot Virus under Field Conditions. SHYI-DONG YEH, Huey-Jiunn Bau, and Li-Fang Chen. Department of Plant Pathology, National Chung Hsing University, Taichung 40227, Taiwan. E-mail: sdyeh@nchu.edu.tw

Field trials of the three transgenic papaya lines carrying the coat protein (CP) gene of *Papaya ringspot virus* (PRSV) were conducted from 1996 to 2000 to evaluate their resistance to PRSV. Breakdowns of the resistance were noticed in the trials of 1999 and 2000. Two virus isolates, 5–19 and DL1, were collected from diseased transgenic papaya of the third and the fourth trials, respectively. When tested by enzyme-linked immunosorbent assay (ELISA), 5–19 reacted strongly with the antiserum to PRSV whereas DL1 was negative. Under greenhouse conditions, 5–19 and DL1 were able to break down the resistance of PRSV CP-gene transgenic papaya lines and induced symptoms on untransformed plants different from those induced by the strain YK, a prevalent mosaic type of PRSV in Taiwan. Using a primer pair specific to PRSV, the CP gene of 5–19 was amplified by reverse transcription-polymerase chain reaction, and subsequently cloned. Sequence analysis revealed that the CP gene of 5–19 shares 96.24% nucleotide identity and 95.09% amino acid identity with that of YK, indicating that it is a PRSV strain diversified from YK. The DL1 isolate was further identified as *Papaya leaf-distortion mosaic virus* (PLDMV) for it reacted strongly with the antiserum to the Okinawa isolate of PLDMV in ELISA. With the primers specific to potyviruses, the CP gene and the 3'-noncoding region of the DL isolate was amplified, cloned, and sequenced. The results showed that the CP gene and the 3'-noncoding region of DL isolate shares 94.9% amino acid identity and 96.2% nucleotide identity, respectively, with those of Okinawa PLDMV. Since the DL isolate did not infect cucurbits, it was concluded that the DL isolate is a new pathotype of PLDMV. The resistance-breaking PRSV strain and the new pathotype of PLDMV are considered as major threats for the application of PRSV CP-gene transgenic papaya lines in Taiwan.

P-1044

Modification of Serine Acetyltransferase Activity as the Way to Study Cysteine Metabolism in Plants. A. BLASZCZYK and A. Sirko. Institute of Biochemistry & Biophysics, Polish Academy of Science, Pawinskiego 5A, 02–106 Warsaw, Poland. E-mail: blaszczyk@ibb.waw.pl

Sulfur plant metabolism is not well known yet. However, biosynthesis of cysteine in plants occurs by a process similar to that known in microorganisms with the activity of serine acetyltransferase (SAT) as bottle-neck. SAT catalyzes production of O-acetylserine (OAS) from serine and acetyl-CoA. Besides hypothetical regulatory function of OAS in transcriptional activation of cysteine biosynthetic pathway, it is the limiting factor for the levels of cysteine and glutathione. Glutathione has multiple functions in plant metabolism—it plays a pivotal role in maintaining the redox homeostasis, regulation of the cell cycle and finally in responses to various environmental stresses. To increase the amount of thiol compounds we expressed bacterial gene encoding SAT in tobacco plants. As a result, we observed elevated cysteine and glutathione levels that correlated well with increased resistance of transgenic plants to oxidative stress. Stability of transgene expression was confirmed in next generations. Previously reported features, like higher thiols contents and oxidative stress resistance appeared to be maintained. Selected transgenic lines of tobacco plants were further biochemically characterized. Several new intriguing observations were made, e.g. elevated total protein content, changes in some enzymatic activities and chemical elements levels. Transgenic plants producing bacterial SAT, that is not regulated by the factors regulating the intrinsic plant SAT, are the good model to study the effects of all potential regulatory factors on cysteine metabolic genes' expression. Our results strongly suggest that SAT activity is a limiting factor for the amount of cysteine and glutathione produced by plants and also may affect other metabolic pathways.

P-1045

Epigenetic Effect of Cold Acclimation on Rice Tissues. S. CASTIGLIONE and T. Fossati. Dipartimento di Biologia, Via Celoria, 26 Università degli Studi di Milano. E-mail: stefano.castiglione@unimi.it

Plant resorts epigenetic variation to respond to environmental modifications and to modulate embryo development. These genetic modifications are commonly inherited, but they can revert to the previous situation, in fact these changes are not mutations of the nucleotide sequence, but modification of the methylation content: addition of a methyl group to the cytosine ring is the most common event. Alteration of the methyl cytosine content in plant and animal genomes can be easily monitored using a molecular approach: the MSAP technique (Methylation Sensitive Amplified Polymorphism—Xiong et al., 1999). These molecular tool is based on different sensitivity of two restriction enzymes *Hpa*II and *Msp*I to methyl-cytosine present on the target DNA sequence. We used this methodology to study the effect of cold treatment on different rice tissues. Meristematic root apexes and full expanded leaves exposed to cold treatment (6° C) for one week were harvested, the DNA of control plants and of treated plants was purified and amplified. MSAPs were observed in the both assayed tissues. Six and twelve MSAP fragments were identified in full expanded leaves and in meristematic root apexes, respectively. This is probably one of the first scientific evidences that abiotic stresses can induce epigenetic variation in rice genome. Xiong LZ et al. Patterns of cytosine methylation in an elite rice hybrid and its parental lines, detected by a methylation-sensitive amplification polymorphism technique (1999) 261, 439–446.

P-1046

Genetic Analysis of Drought Resistance in Rice by Molecular Markers. R. CHANDRA BABU[1], Varapong Chamarerk[2], P. Shanmugasundaram[1], P. Jeyaprakash[3], S. Sadasivam[1], and H. T. Nguyen[2]. [1]Centre for Plant Molecular Biology, Tamilnadu Agricultural University, Coimbatore – 641 003, India; [2]Dept. of Plant & Soil Science, Texas Tech University, Lubbock, TX; and [3]Agricultural Research Station, Tamilnadu Agricultural University, Paramakudi, India. Email: chandrarc@hotmail.com

Quantitative trait loci (QTL) linked to plant water relations, phenology, and production traits under irrigated and drought stress conditions were mapped using a doubled-haploid (DH) population of 154 rice (Oryza sativa L.) lines. A total of 48 putative QTLs were identified for various plant water relations and production traits under control and water stress conditions in the field, and individually explained 4.8 – 58.8% of the phenotypic variation. All the traits exhibited not only putative main-effect QTLs but also putative epistatic effect QTLs. The putative epistatic QTLs lying between RG449-EMP3_1c on chromosome 4 interacted with both the environments tested and produced significant contribution to the total phenotypic variation for yield under stress. The nature of association between drought resistance components (root traits and capacity for osmotic adjustment) and rice production under drought was studied using coincidence of QTLs. Root traits had positive correlations with yield under stress. The region, RG939-RG476-RG214 on chromosome 4 identified for root trait also had pleiotropic effect on yield under stress. Consistent QTLs for drought resistance traits and yield under stress were detected and should be useful for marker-assisted selection for rainfed rice improvement.

P-1047

Isolation and Characterization of Low Temperature-Responsive Promoters in *Poncirus trifoliata* (L.). KAREN I. CHAMP and Gloria A. Moore. Horticultural Sciences Department, Plant Molecular and Cellular Biology Program, University of Florida, Gainesville, FL 32611. E-mail: KAREN@UFL.EDU

We have isolated 5' genomic DNA sequence for 4 *Poncirus trifoliata* (L.) genes upregulated in response to low temperature using inverse PCR. Genomic sequences were analyzed using both PLACE [Higo et al., NAR 27(1):295–296] and PlantCARE [Rombauts et al., NAR 27(1):297–300] computer databases to identify putative cis-elements responsible for cold activation. The databases predicted low-temperature response elements (LTREs) similar to those in *Arabidopsis thaliana* and *Hordeum vulgaris*. Dehydration stress and abscisic acid (ABA) have been shown to be important in the regulation of low temperature-regulated genes in other species, and multiple drought and ABA-responsive elements (ABREs) were also putatively identified. Notably, the 5' sequence of CORc115 and CORc410 contain the core (CCGAC) of the LTRE of *A. thaliana* COR15a, a gene upregulated by the CBF gene family in response to low temperatures. In addition, CORc102 and CORc410 contain 1 and 2 copies, respectively, of the *H. vulgaris* blt4.9 LTRE core (CCGAAA). Current research is focused on using *Agrobacterium*-mediated transient transformation to determine which promoter elements are functionally important for gene upregulation upon exposure to low temperatures *in vivo*. Positive identification of these sequences will facilitate the isolation of low temperature signaling components in *Poncirus* and economically important *Citrus* species.

P-1048

Overexpression of *Arabidopsis* DREB1B Confers Water-deficit Tolerance to Transgenic Tomato. Tsai-Hung Hsieh, Yee-yung Charng, and MING-TSAIR CHAN. Insititute of BioAgricultural Sciences, Academia Sinica, Taipei, 11529, Taiwan. E-mail: mbmtchan@ccvax.sinica.edu.tw

The *Arabidopsis DREB1B* cDNA, driven by CaMV 35S promoter, was transformed into tomato genome by *Agrobacterium*-mediated method. Transgenic tomato plants were identified by GUS staining, Southern, and Northern blot analyses. The tolerance of transgenic tomato plants against water deficit stress was revealed by measuring survival rate, Fv/Fm values, and relative water content. In addition, the transgenic tomato plants contained high level of proline than that of the wild-type plants under normal or water deficit condition. Subtractive hybridization was used to isolate the responsive genes of heterologous *DREB1B* in transgenic tomato plants, and the *CAT1* was first characterized. The increase of *CAT1* transcripts and catalase activity, and reduction of H_2O_2 concentration were observed in transgenic tomato comparing to wild-type plants. All of these results showed that the transgenic tomato plants were more tolerant to water deficit stress than wild-type plants. The gibberellin 3 (GA_3) treatment exogenously can reverse the growth retardation of transgenic tomato plants, however, the water deficit tolerance was not affected. These results indicated that the heterologous *Arabidopsis DREB1B* could confer water deficit tolerance in transgenic tomato plants.

P-1049

Development of a Mature Embryo Culture System for Wheat Cultivars. SANJAY VELANDY CHODAPARAMBIL[1], S. Ganeshan[1], D. B. Fowler[2], and R. N. Chibbar[1]. [1]National Research Council, Plant Biotechnology Institute, Saskatoon, SK, S7N 0W9, Canada and [2]Crop Development Center, University of Saskatchewan, 51, Campus Drive, Saskatoon, SK, S7N 5A8, Canada. E-mail: RAVI.CHIBBAR@NRC.CA

The main objective of this study is to optimize regeneration systems for wheat from mature embryos. Five commercial cultivars of wheat were used, *viz.,* CDC Osprey, CDC Clair, CDC Ptarmigan (winter wheats), Plenty (durum wheat) and AC Nanda (soft-spring wheat). Different media formulations based on Murashige-Skoog (MS) medium and use of different plant growth regulators such as cytokinins have been tested with the above-mentioned cultivars to assess their responses for the production of multiple shoots directly, without an intervening callus phase. This novel system is advantageous, since it does not require growth of donor plant materials and mature seeds are always available. Our preliminary data indicate that, depending on the cultivars, 3–9 shoots can be regenerated per mature embryo just after one sub-culture and we foresee a more improved shoot production after the second sub-culture. Such mature embryo explants will be used as target tissues for delivery of foreign DNA and to assess the expression of the gene(s) of interest. In this preliminary study we have been able to demonstrate the induction of multiple shoots from mature embryos at a high frequency. Our studies indicate that this system would be of interest to explore further and be assessed for its efficiency in transformation studies using microprojectile bombardment and/or *Agrobacterium tumefaciens* mediated DNA delivery.

P-1050

Biochemical Characteristics of Transgenic Tobacco Plants and Cells Expressing Human Dehydroascorbate Reductase Gene. SUN-MEE CHOI, Suk-Yoon Kwon, Haeng-Soon Lee, and Sang-Soo Kwak. Plant Cell Biotechnology Lab., Korea Research Institute of Bioscience and Biotechnology (KRIBB), Yusong, Taejon 305–600, KOREA. E-mail: sskwak@mail.kribb.re.kr

Dehydroascorbate reductase (DHAR; EC 1.8.5.1) is an enzyme catalyzes the reduction of dehydroascorbate (DHA) to ascorbic acid (AsA, ascorbate). To analyze the physiological role of DHAR in environmental stress adaptation, we developed transgenic tobacco (*Nicotiana tabacum* cv. Xanthi) plants and cells expressing a human DHAR gene in chloroplasts and investigated their protection effect to oxidative stress induced by methyl viologen (MV), hydrogen peroxide (H_2O_2), low temperature and salt. DHAR activity and AsA content in DHAR transgenic plants were 1.43 and 1.8 times higher than those of non-transgenic (NT) plants, respectively. When tobacco leaf discs were subjected to MV (5 ¥ıM) and H_2O_2 (200 mM), DHAR transgenic plants showed 40% and 25% less cellular damage than NT plants, respectively. The enhanced tolerance to low temperature (15° C) or salt (100 mM NaCl) stress was also observed in transgenic plants. In addition, DHAR transgenic tobacco cell lines were successfully induced from the leaf tissues of DHAR plants on MS medium containing 2 mg/L NAA and 0.1 mg/L BA to understand the physiological function of DHAR in cultured cells. DHAR activity in transgenic callus was about 1.53 times higher than that of NT cell lines. DHAR transgenic cell lines showed an increased tolerance to MV-mediated oxidative stress in suspension cultures. Theses results suggest that a human DHAR cDNA was properly worked in cultured cells as well as whole plants.

P-1051

Isolation and Characterization of Phosphatidylinositol Synthase (PIS), Phosphatidylinositol-specific Phospholipase C (PI-PLC) and Phosphatidylinositol 3-Kinase (PI3-K) from *Brassica napus*: Their Expression in Various Tissues and Under Different Abiotic Stress Conditions. SHANKAR DAS, Atta Hussain, and Fawzy Georges. Plant Biotechnology Institute, National Research Council of Canada, 110 Gymnasium Place, Saskatoon, SK., Canada S7N 0W9.

In order to detect and react to signals caused by external stimuli through a specific intracellular response, plants must continuously monitor both biotic and abiotic environments in which they grow. The best characterized signaling network in plants to various stresses, such as drought, salt and cold, is mediated by the phosphoinositide-specific (PI-specific) signal transduction pathway (Munnik et al., 1989; MunniK et al., 1998; Munnik et al., 2001). Phosphoinositdes are components of the membrane in both plants and animals. Their biosynthesis starts with the formation of phosphatidylinositol (PI) by uniting CDP-diacylglycerol and inositol as substrates catalyzed by the enzyme phospahtidylinositol synthase (PIS). PI is further phosphorylated to PI 4-monophophate (PIP) and PI 4,5-bisphosphate (PIP_2) by two other enzymes. The most widely studied polyphosphoinositide (PPI) in this pathway is PIP_2. The latter is hydrolyzed by phosphoinositide-specific phospholipase C (PI-PLC) to produce diacylglyceride (DAG) and IP_3. Both of these compounds are second messengers in the signal transduction pathway. Further phosphorylation of PIP_2 by PI3-kinase leads to PI 3,4,5-trisphosphate (PIP_3). Recent evidence showed that most of these enzymatic steps respond to various stresses (Hirayama et al., 1995; Mikami et al., 1998; Meijer et al., 1999; Pical et al., 1999; Takahashi et al., 2001). We, for the first time, have isolated and characterized three genes PIS, PI-PLC and PI3-kinase from Brassica napus, a most important oilseed crop in many countries including Canada. We also studied the expression pattern of these genes in different tissues and under abiotic stress conditions such as drought, salt and cold. The results are discussed in this presentation.

P-1052

Isolation and Characterization of rd22 (Responsive to Dehydration) Gene from Potato and Transformation of Tobacco with the rd22 Gene. Hye-Sook Song, Dool-Yi Kim, Jae-Bok Park[1], Shin-Chul Bae, SEUNG-JOO GO, and Myung-Ok Byun. Molecular Genetics Division National Institute of Agriculture Science and Technology, RDA, Suwon, 441–707, Korea. [1]Dept. of Environmental Horticulture, University of Seoul, Seoul, 130–743, Korea. Email: sjg@rda.go.kr

The freezing tolerance is one of the major targets for the future agriculture. Biotechnology has the potential to improve the freezing tolerance of crops by transgenic plant technology. The limiting factors for developing this technology are the isolation of genes those active function in freezing tolerance and the precise understanding of the molecular process of freezing tolerance. In order to analyze the response of plants to freezing stress at the molecular level, several cDNA clones which are related to freezing tolerance were isolated from Solanum tuberosum L. and were compared with other cDNA clones' expressions under stressed conditions. The isolated full-length potato RD22 cDNA consists of 1,120 bp nucleotides, containing an open reading frame of 1,092 bp, which encodes a polypeptide of 364 amino acids with a molecular mass of about 39 kDa. RD22 gene showed 59% and 74% homology to Arabidopsis thaliana RD22 gene at DNA and protein level, respectively. For functional analysis of RD22 gene using transgenic plants, a pBIN121 vector containing RD22 gene was constructed for transformation in which RD22 gene was driven by the Arabidopsis thaliana rd29A promoter. By Agrobacterium-mediated transformation method, several RD22 transgenic tobacco plants were obtained. The transgenic tobacco plants were analyzed by Southern and Northern blot analyses to confirm insertion and expression of RD22 gene in the transgenic plants. Corresponding Author: mobyun@rda.go.kr

P-1053

Mitigation of Transgene Flow from Crops to Related Weeds. H. AL-AHMAD and J. Gressel. Plant Sciences, Weizmann Institute of Science, Rehovot 76100, Israel. E.mail: alahmad@wicc.weizmann.ac.il

Some transgenic crops can interbreed with related weeds, increasing the potential of the hybrid progeny for competition. Crops themselves can become volunteer weeds in the subsequent crop, or even become feral, re-evolving the traits of weed progenitors. Transgenetic Mitigation (TM), where a primary gene is coupled in tandem with TM genes that are positive or neutral to a crop but deleterious to related weeds, can be used as a failsafe mechanism to mitigate the effects of transgene transfer from crops to related species. The overall effect would reduce the competitive ability of the rare transgenic hybrids, so that they can no longer persist in agroecosystems. In this study, a primary *ahas* gene (*csr 1.2*) conferring resistance to herbicide inhibition of acetolactate synthase has been cloned in a tandem construct with a transgenetic mitigator 'antiweediness' gibberellic acid-insensitive semidominant dwarfing mutant gene (*gai*). Dwarfing is disadvantageous for weeds that can no longer compete with cohorts or the crop, but is desirable in many crops, preventing lodging and increasing the harvest index. The tandem genes were transformed into tobacco via *A. tumefaciens* and were expressed in seven independent transformed lines. The semi-dwarf transformants regenerated normally in the presence of 0.3 μM imazapyr, which suppressed the non-transgenic controls. The presence of linked *ahas* and *gai* genomic inserts in the seven lines was confirmed by PCR amplification. All the T_0 plants were male sterile, but they set seeds normally when pollinated from untransformed control plants. The $T_1(=BC_1)$ progeny segregated according to a typical Mendelian ratio (1:1, linked imazapyr resistant and dwarf to imazapyr sensitive and tall). The TM transgenic plants remained dwarf and could not compete with the wild-type in an ecological competition experiment between segregating wild-type and $T_1(=BC_1)$ tobacco plants.

P-1054

Osmotin-role in Cell Death Pathway. GOHAR TAJ KHAN[1], G. K. Garg[1], and K. C. Bansal[2]. [1]Molecular Biology and Gen. Eng. Department, G. B. Pant University, Pantnagar, India and [2] National Research Center on Plant Biotechnology, Indian Agricultural Research Institute, New Delhi, India. Email: gohartajkhan@rediffmail.com

Environmental stresses such as salt and drought are among the factors most limiting to plant productivity. Such stresses are becoming even more prevalent as the intensity of agriculture increases. Therefore, elucidation of mechanisms by which plants perceive and signal the plant about these stresses is crucial if we are to understand the plant response and introduce the genetic or environmental improvement to stress tolerance. Osmotin, a stress protein has been engineered in *Brasica juncea* and its influence was studied. Transformed calli were subjected to different concentration on NaCl (50–200 mM). The calli were studied for changes in morphology, growth, changes in expression of different signal transduction proteins, osmotin, p53, caspase 1, caspase 3, cycline B, CDC by ELISA and accumulation of proline, free radical and H_2O_2 by biochemical methods. All concentration resulted in increase of cell death protein (p53, caspase1 and caspase3) and suppression of cell survival protein (cycline B and CDC). NaCl induced the cell death pathway through p53, caspase 1 and caspase 3. Osmotin perturbed the signal transduction and gave amore complex picture. Osmotin was suppressed the p53 only and not other cell death proteins. The results harbor the significance to understand the signaling pathway.

P-1055

Influence of Biotic Stress on the Plant Genome. IGOR KOVALCHUK. University of Lethbridge, 1109 Elliot Road S., Lethbridge, Alberta, T1K 3V2, Canada. Email: igor.kovalchuk@uleth.ca

Plant genome stability is known to be influenced by many environmental abiotic factors(radiation, cold, draught, etc.). In contrast, molecular data on influence of pathogens on the host genome are so far lacking. In order to study the genome stability of plants, transgenic tobacco carrying the vital luciferase-based marker gene serving as homologous recombination substrate were generated. Our transgenic plants had already proved to be sufficiently sensitive to detect a systemic increase in homologous recombination in response to local abiotic stress treatments (rose bengal). These results pointed to the existence of cross-talk between exposed and non-exposed parts of the plant. Thew sensitivity of a system able to efficiently monitor the mutagenic influence of abiotic factors seemed satisfactory for the study of the influence of biotic stress. For plant pathogen interactions, we have chosen tobacco-tobacco mosaic virus (TMV) interaction. Here we report that TMV infection causes genome instability; infected plants exhibit three-fold increase of homologous recombination frequencies. Grafting experiments demonstrated that the recombination increase also occurred in non-inoculated tissue and was due to a plant signal generated in infected cells. The recombination-inducing signal was pathogen related, as mechanic wounding did not trigger the production of the signal. Our experiments suggest that biotic stress leads to the genome rearrangement via systemic induction of the homologous recombination, a specific mechanism of plant defense. We discuss potential mechanisms for generation of the signal and possible adaptive advantages of enhanced genomic flexibility following virus infection.

P-1056

Selection and Characterizations of Radiation Induced Saline Tolerant Lines via Rice Embryo Culture. Y. I. LEE, I. S. Lee, D. S. Kim, D. Y. Hyun, I. C. Shin, and Y. P. Lim*. Radiation Genetic Resources Dept., Korea Atomic Energy Research Institute, P.O. Box 105. Yuseong, Taejon 305–600 and *Department of Horticulture, Chungnam National Univ., Korea. E-mail: yilee@nanum.kaeri.re.kr

To select a saline tolerant rice mutants, NaCl tolerant cell lines were selected from the callus irradiated with gamma ray through embryo culture. The callus was subcultured at every 4 week interval on the M&S medium containing 1.5% NaCl and selected at the time of 120 days after inoculation. Regenerants were obtained from the tolerant callus around 60 days of culture on the auxin-free medium. The regenerants were screened again in 1.5% NaCl solution for confirming the inheritance of the tolerance from callus to regenerant. M2 seeds were harvested from the screened regenerants. The sterility of M1 plants were much increased with increasing radiation doses. Four salt tolerant lines were selected among 350 lines regenerated from tolerant calli. The agronomic traits of selected lines were increased compared to original variety such as plant height, panicle length, tillering, grain weight and yield production under cultivation on 0.8% saline paddy field. The contents of proline, phenolic compounds and sugar was increased in the saline tolerant lines than those of original Dongjinbyeo. The activities of H+-ATPase, peroxidase and catalase in salt tolerant lines were increased. However, starch content and EC values were decreased in the tolerant lines. Twelve AFLP primer combinations produced a total of 661 bands with an average of 55 per reaction. The number of bands for each primer varied from 41 in AAG+CAA to 61 in AAG+CAC. Total polymorphism rate in mutants were 18% (120 out of 661 bands) compared to original plant. A specific band generated in ACG+CTG primer combination was only detected in the salt tolerant lines, but not in original plant. The AFLP band has homology with a rbcL gene of Poaceae and cold stress-induced cDNA of soybean related to salt tolerance.

P-1057

Selection of Azetidine-2-Carboxylic Acid Resistant Lines by In Vitro Mutagenesis in Rice (*Oryza sativa* L.). Y. I. LEE, D. Y. Hyun, I. S. Lee, D. S. Kim, S. J. Lee, and Y. W. Seo*. Radiation Genetic Resources Dept., Korea Atomic Energy Research Institute, P.O. Box 105. Yuseong, Taejon, 305–600, Korea and *Division of Biotechnology and Genetic Engineering, Korea Univ., Seoul, 136–701, Korea. E-mail: yilee@kaeri.re.kr

The resistant cell lines to azetidine-2-carboxylic acid (AZC) were selected through rice embryo culture after mutagenic treatment of callus with gamma rays. The optimum AZC concentration for the selection of resistant cell lines was 3 or 4 mM AZC considering LD_{50} and fresh weight of callus. Survival rate of the AZC resistant callus was remarkable increase in the callus irradiated with 50 and 70 Gy. Regeneration rate of the AZC resistant callus was much lower on the whole. Putrescine (Put) was effective to generate greenspot. Callus on the medium of 500 mM Put showed the best proliferation and vigour. Cross resistance to NaCl and PEG showed elevated resistance for osmotic stress due to gamma rays. The level of free proline content in the AZC resistant callus was increased up to 2.3 times compared to control callus. Proline content in the regenerants derived from AZC resistant callus also increased to 1.7 times than control plants that were regenerated from callus grown in AZC free medium. However, increase of proline in the regenerants was much lower than in callus. The results of RAPD profile showed polymorphism of 12.4 and 11.2% in calli and regenerants, respectively. One primer (OPH-06) generated specific fragment of 0.6 Kbp, which was only present in the resistant cell lines. AFLP patterns demonstrated that polymorphism of the callus was much higher than that of regenerants. Polymorphic products from resistant callus treated with gamma ray and regenerants from the callus showed high variation according to increasing radiation dose. This result indicated that mutagenesis with gamma rays might be an effective tool to *in vitro* selection for AZC resistant lines.

P-1058

Physiological Responses and Secondary Metabolism of Plant Cells Induced by Low-Energy Ultrasound. LIDONG LIN and Jianyong Wu. Department of Applied Biology and Chemical Technology, The Hong Kong Polytechnic University, Hung Hom, Kowloon, Hong Kong. E-mail: 99900119r@polyu.edu.hk

Ultrasound (US) is a special type of physical stimulus that has shown a range of bioeffects in various biological systems. In this work, we examined the effects of low-energy US on the secondary metabolite biosynthesis of plant cells in suspension cultures and the mechanism of the effects. Experiments were carried out in three plant cell culture systems, *Panax ginseng*, *Lithospermum erythrorhizon* and *Taxus chinensis*. The cell suspension cultures were exposed to US at power density below 82 mW/cm^3 for short periods (1–8 min). Under most exposure conditions, US stimulated the biosynthesis of secondary metabolites, increasing saponin yield of *P. ginseng* and shikonin yield of *L. erythrorhizon* by 60–70%, and taxol yield of *T. chinensis* by 4-fold. The effects of US showed a significant correlation with the total US energy applied. The activity of some enzymes related to the secondary metabolite production of the cells, e.g., phenylalanine ammonia-lyase (PAL) and geranyltransferase for shikonin, were increased by the US treatment. The enhancement of plant cell secondary metabolism by US appears to be a result of ultrasound-induced defense response of the plant cells, as some plant defense-related reactions, e.g., the oxidative burst and enzymatic browning have been detected. Other physiological effects of US found in our work include increase in the intracellular product release and the membrane permeability, decrease of cell volume and water content. In this paper, we will show these novel effects and discuss the underlying mechanisms.

P-1059

Characterization of Transgenic Tobacco Producing Bacterial Enzymes of the Cysteine Biosynthesis Pathway. F. LISZEWSKA, A. Blaszczyk, A. Sirko. Institute of Biochemistry & Biophysics, Polish Academy of Sciences, Pawinskiego 5A, 02–106 Warsaw, Poland. E-mail: frantz@ibb.waw.pl

Biosynthesis of cysteine in plants constitute the final step of sulfur assimilation from inorganic sulfate into organic molecules. It occurs by a process similar to that in bacteria. Formation of cysteine from sulfide and *O*-acetyl-L-serine (OAS) is catalyzed by *O*-acetylserine (thiol) lyase (OAS-TL) while OAS is synthesized by serine acetyltransferase (SAT) from acetyl-coenzyme A and serine. Availability of OAS, depending exclusively on the SAT activity, is the limiting element in the biosynthetic flow of cysteine, whose amount influence positively the subsequent synthesis of glutathione. Molecular interactions between SAT and OAS-TL are involved in the regulation of the enzymatic activities of these proteins in both bacteria and plants. The stability of the complex is negatively affected by OAS and positively by sulfide. Transgenic tobacco plants with cytosolic production of either SAT, OAS-TL or both were analysed for thiols levels and physiological consequences of the transgenes expression. We demonstrated that in the *in vivo* conditions, overproduction of either SAT or OAS-TL in the cytosol had an influence on sulfur flow and accumulation of the thiol metabolites. Overproduction of SAT resulted in a much more significant increase of cysteine and glutathione and enhanced resistance to oxidative stress generated by hydrogen peroxide than overproduction of OAS-TL. Simultaneous production of bacterial OAS-TL and SAT resulted in slightly increased thiols contents as compared to single SAT-overproducers. We presently report the biochemical characteristics of the single and double transgenic progeny of double transformants.

P-1060

MP2C—Medicago Phosphatase 2 C Interacts with Mitogen Activated Protein Kinases (MAPKs) In Vivo and In Vitro. ANETA LIWOSZ, Emmanuel Baudouin, Alois Schweighofer, Irute Meskiene, Heribert Hirt. Institute of Microbiology and Genetics, Vienna Biocenter, University of Vienna, 1030 Vienna, Austria. Email: aneta@gem.univie.ac.at

Protein phosphatases play important roles in eucaryotic signal transduction. They are classified into three major classes: serine/threonine, tyrosine and dual specificity phosphatases. MP2C belongs to the serine/threonine class of protein phosphatases of type 2C and occurs as a monomer with a highly conserved catalytic domain and a characteristic N-terminal extension. The N-terminal part of MP2C contains a MAPK-docking motif which is conserved in several MAPK—interacting proteins. Recently, screening of a yeast two hybrid cDNA library from Medicago sativa, we have found SIMK (Salt Stress—Induced MAPK) as an interacting partner for MP2C. In vitro binding data also have shown that MP2C binds to SIMK. Structure—function analysis revealed that N-terminal part of MP2C is responsible for binding with SIMK. MP2C also dephosphorylates and inactivates SIMK in vitro. Our in vitro and in vivo data indicate that MP2C is a MAPK phosphatase functioning as a negative regulator of SIMK.

P-1061

Maize Genotypic Variation on Tolerance to Osmotic Stress In Vitro. C. LÓPEZ-PERALTA, L. Iracheta-Donjuan, V. A. González-Hernández, and E. Cárdenas-Soriano. Laboratorio de Biotecnología Agrícola, IREGEP-Colegio de Postgraduados, km. 36.5 Carr. México-Texcoco, Montecillo, Edo. de México. México. CP 56230. E-mail: cristy@colpos.mx

The level of tolerance to *in vitro* osmotic stress induced by PEG-8000 on the *in vitro* organogenesis of four maize genotypes (3 inbred lines and one hybrid) was evaluated during the multiplication phase. The basal liquid medium (Murashige & Skoog, 1962) was supplemented with BAP (6.66 μM) and IAA (5.70 μM); PEG-8000 concentrations varied from 0 to 30% (w/v), to produce 13 levels of osmotic stress. As expected, the organogenic capacity of maize was increasingly diminished by increments in PEG concentration in the medium, in the four genotypes, as well as the shoot height and survival. However, the effect of osmotic stress varied among genotypes and among variables of response, since line L13 was the most tolerant regarding shoot survival, whereas line L6 produced the highest number of shoots per explant when subjected to stress. The lethal dose (LD_{50}) for the number of shoots per explant varied between 12.5 and 15% of PEG-8000.

P-1062

In Vitro Organogenesis of Maize Under Osmotic Stress. C. LÓPEZ-PERALTA, J. Mejía-Carranza, P. Ramírez-Vallejo, and E. Cárdenas-Soriano. Laboratorio de Biotecnología Agrícola, IREGEP-Colegio de Postgraduados, km 36.5 Carr. México-Texcoco, Montecillo, Edo. de México. México. CP 56230. E-mail: cristy@colpos.mx

In vitro plant tissue culture could be a helpful tool to the techniques of conventional improvement applied for identification and selection of osmotic stress tolerant lines. The aim of this research was to evaluate the tolerance of shoots obtained by organogenesis *in vitro* of three lines (L 16, L 13 and L19) and a hybrid (SJ10x11), from lateral buds of tissue cultivated during three cycles in constant osmotic stress conditions induced with 12% polyethylene glycol (PEG, M.W. 8000) and one more cycle in wich seven different concentrations (7.5 to 20%) of the same non penetrating osmolite were assessed. Results showed an increase in the tolerance indices for bud multiplication (69%) and height of plantlets (129%). Differences in response to multiplication and quality of plantlets between genotypes were obtained because of genetic variability. Osmotic adjustment was not sufficient to keep plantlets alive when they were cultivated under the effect of PEG-8000. Histological analysis showed that the buds were originated from the lateral shoots of the initial plantlet; besides, there was starch accumulation in meristematics zones specially in buds under osmotic stress, probably due to osmotic adjustment. The results suggest that the increase in levels of PEG could be useful to obtain germoplasm tolerant to osmotic stress.

P-1063

The Identification of CBF Homologs in Palms. L. LU, M. J. Glueckert, E. Nagy, D. A. Francko, and K. G. Wilson. Department of Botany, Miami University, Oxford, OH 45056. E-mail: luwangming@hotmail.com

Several palm varieties are naturally resistant to freezing temperatures even though they are predominantly tropical to subtropical plants. Based on our field experiments, many species of palms, such as *Rhapidophyllum hystrix, Sabal minor, S. palmetto* and others, can survive freezing temperatures approaching 0° F and even lower with little or no foliar damage. The mechanism of this cold resistance is unknown. Thomashow and coworkers have demonstrated that *CBF* genes, a small family of regulatory genes in *Arabidopsis thaliana*, are important in the plant cold response. After plant exposure to low nonfreezing temperatures, the *CBF* genes are quickly induced and in turn up-regulate a series of cold-related genes. The *CBF*-like genes have also been reported in *Brassica napus*, rye, wheat, and tomato. We have identified possible *CBF* homologs from cDNAs and genomic DNAs of known cold-resistant palms. We report on the sequences of these *CBF*-like genes and their expression patterns under cold stress. The functions of these genes in palm cold tolerance will be discussed, along with potential applications to commercial palm propagation.

P-1064

Evaluation of a Technique to Measure Plant Cell Viability Using Image Analysis. L. Miranda-Hernández, G. Trejo-Tapia, A. Jiménez-Aparicio, E. Galindo*, Mainul Hassan*, and MARIO RODRÍGUEZ-MONROY. Dep. de Biotecnología. CEPROBI-IPN. PO. Box 24. Yautepec, Morelos. México 62731 and *Dep. Bioingeniería. IBT- UNAM. PO. Box. 510–3. Cuernavaca, Morelos, México 62250. E-mail: mrmonroy@ipn.mx

Cell viability is fundamental to define the best conditions to grow plant cells in bioreactors, as well as for cryopreservation studies. Fluorescein diacetate (FDA) is used frequently as a vital dye and the cells are analyzed under a microscope, this technique is subjective and depends of the analyst considerations. Image analysis should be considered as a powerful tool to determine cell viability, as well as other cell culture characteristics like cell morphology. This study proposes a new methodology to determine plant cell viability using FDA and image analysis. *Beta vulgaris* cultures were stained with FDA (5 μg/ml), the fluorescent cells were stable to ilumination in UV light, which allowed digital image acquisition. Cell viability was expressed as the percentage of FDA stained plant cell area over total plant cell area. In order to evaluate the effect of different damage levels in the development of cultures, *B. vulgaris* cells were subjected to radiation (160 Watts) and heat (65° C). In both treatments, cell viability decreased as exposure time increased. At the same time, samples were cultivated in shake flasks in order to evaluate plant cell growth and to calculate the specific growth rate in the different samples for assessing viability in terms of plant cell growth performance. The viability of treated cells measured by image analysis correlated linearly with the specific growth rate.

P-1065

Isolation and Characterization of a Novel Stress-inducible Antioxidant Enzyme in the Resurrection Plant *Xerophyta viscosa*. S. B. MOWLA, J. A. Thomson, J. Farrant, and S. G. Mundree. Plant Stress Research Unit, Department of Molecular and Cell Biology, University of Cape Town, South Africa. E-mail: Shaheen@molbiol.uct.ac.za

A cDNA corresponding to 1-Cys peroxiredoxin, an evolutionary conserved thiol-specific antioxidant enzyme, was isolated from *Xerophyta viscosa* Baker, a resurrection plant indigenous to Southern Africa and belonging to the family *Vellociaceae*. The cDNA, designated *XvPer1*, contains an open reading frame that encodes a polypeptide of 219 residues with a predicted molecular weight of 24.2 kDa. XvPer1 polypeptide shows a high level of sequence identity (about 70%) to other recently identified plant 1-Cys peroxiredoxins and relatively high levels of sequence similarity with non-plant 1-Cys peroxiredoxins. The *XvPer1* cDNA contains a putative nuclear localization signal (NLS) as well as a putative polyadenylation site. As for all 1-Cys peroxiredoxins identified to date, the amino acid sequence proposed to constitute the active site of the enzyme (PVCTTE) is highly conserved in XvPer1. Southern Blot analysis has revealed that there is a single-copy of *XvPer1* in the *X. viscosa* genome. All plant 1-Cys peroxiredoxins described to date are seed-specific and absent in vegetative tissues under any condition. The *XvPer1* transcript is unique in that it is expressed in the vegetative tissues of *X. viscosa*. The *XvPer1* transcript is absent in fully hydrated *X. viscosa* tissue but transcript level increased in tissues subjected to abiotic stresses such as dehydration, heat, high light intensity and when treated with abscisic acid (ABA) and sodium chloride (NaCl). To investigate the behaviour and function of the XvPer1 protein, the transcript was cloned into a prokaryotic protein expression vector and expressed in *E. coli* and thereafter purified using Ni-NTA agarose beads. The purified protein was used to raise polyclonal antibodies in rabbits. Western blot analysis was carried out and the results obtained correlated with the patterns of expression of *XvPer1* transcript under different stress conditions. For localization studies, immunofluorescence analysis was performed and showed that XvPer1 is localized in the nucleus of dehydrated *X. viscosa* leaf cells. These results suggest that *XvPer1* is a stress-inducible gene which may function to protect nucleic acids within the nucleus against oxidative injury.

P-1066

Molecular Cloning and Expression of the Stress-Responsive eIF1 Gene in *Porteresia coarctata* Tateoka. LATHA RANGAN***, J. Bennett**, S. G. Hosseini**, M. S. Swaminathan*, M. C. Elliott***, and N. W. Scott***. *M. S. Swaminathan Research Foundation, 3rd Cross Street, Taramani Institutional Area, Chennai, India 6000113; **International Rice Research Institute, MCPO Box 3127, 1271 Makati City, Los Banos, Laguna, Manila, Philippines; and ***Norman Borlaug Institute for Plant Science Research, De Montfort University, Scraptoft, Leicester, LE7 9SU, U.K. E-mail: LRangan@dmu.ac.uk

The cDNA for Translation Initiation Factor 1 (PceIF1) was isolated from the leaves of *Porteresia coarctata* that had been subjected to high salt treatment. Northern analyses showed that the abundance of *eIF1* transcripts initially increased under salt stress of 150 mM sodium chloride and then declined to control levels after 10 days of stress. The gene was also upregulated upon treatment with ABA (20 μM) and mannitol (700 mM). This suggests that induction of the gene is related to the water-deficit consequent upon high salt levels rather than an ion toxicity effect. In spite of the great abundance of eIF1 mRNA in the leaves of salt-treated *P. coarctata* plants, the eIF1 protein could not be detected by Western analysis using anti-TrpE-Sui1 (eIF1) protein antibodies. However levels of *eIF1* transcripts are a convenient marker for monitoring a stress-response mechanism that operates in the leaves of *P. coarctata*. The gene was mapped to the long arm of chromosome 7 of rice using double haploid (DH) mapping populations of IR64 and Azucena.

P-1067

The Search for Molecular Markers Associated with Cold Tolerance in Blueberry. L. J. ROWLAND, S. Mehra, and E. L. Ogden. Fruit Laboratory, Henry A. Wallace Beltsville Agricultural Research Center, U.S. Department of Agriculture, Agricultural Research Service, Beltsville, MD 20705. E-mail: rowlandj@ba.ars.usda.gov

The blueberry industry in the United States suffers significant losses every year due to freezing stress. In fact, an increase in cold hardiness has been identified as one of the major needs of the blueberry industry. To address this need, our laboratory has been using two distinct but related approaches to identify molecular markers/genes associated with cold tolerance in blueberry. One approach has been to map QTLs controlling cold hardiness in the cold acclimated state. Progress toward mapping QTLs will be presented including: (1) construction of initial, low density genetic linkage maps for two diploid (V. darrowi x V. corymbosum-derived) blueberry populations segregating for cold hardiness; (2) use of PCR-based markers including RAPD and more recently EST markers for mapping purposes; (3) evaluation of the mapping populations for cold hardiness; and (4) genetic analyses of the cold hardiness data. The other approach has been to identify, isolate, and characterize cold-responsive genes from blueberry and to map these genes to determine if any map to QTLs that control cold hardiness. Levels of a group of dehydrin proteins (proteins induced by dehydration stress such as freezing and drought) of 65, 60, and 14 kDa increase during cold acclimation such that they become the most abundant proteins in blueberry floral buds during the winter. Progress using a systematic molecular genetic approach will be presented including: (1) characterization of expression of the dehydrins, (2) cloning members of the dehydrin gene family, (3) mapping members of the dehydrin gene family and (4) segregation of the dehydrin genes with the cold hardiness trait.

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Genes Associated with Water Stress Tolerance in Rice Roots. S. SADASIVAM, M. Raveendran, J. A. J. Raja, and R. Chandra Babu. Center for Plant Molecular Biology, Tamil Nadu Agricultural University, Coimbatore, 641 003, India. Email: sadacpmb@hotmail.com

Nootripathu, a rice landrace traditionally grown in rain-fed upland ecosystem having desirable root characters to avoid drought was chosen to identify genes associated with water stress tolerance using differential display (DD RT PCR) technique. Total RNA isolated from roots of Nootripathu subjected to non-stress (irrigated) and drought stress conditions was used for DD RT PCR using a set of 3 anchored primers and 16 arbitrary primers. The differentially expressed cDNAs (80 fragments) were identified by autoradiography and eluted. As a preliminary confirmation of differential expression of these DDRT-PCR products, 25 were identified positive by reverse northern hybridization experiment. The positive fragments were purified and cloned. In order to confirm their differential expression, northern hybridization analysis was performed. The six clones proved to be differentially expressed were sequenced. The sequence analysis showed partial or very less homology with already reported sequences in the database. The 5' coding region of the mRNA corresponding to two differentially expressed partial cDNA was generated by 5' RACE (Rapid Amplification of cDNA Ends). The obtained cDNA's were cloned and sequenced. The sequence analysis revealed the homology of one cDNA with "HSP 90" protein family. The other cDNA clone was found to encode for a hypothetical protein (Mol. Wt. 10 kDa) rich in alanine, proline and leucine. The two proteins encoded by these cDNAs are supposed to play a role in protection of cellular structures by osmotic adjustment and protein recovery which may be one among the reasons responsible for increased tolerance of Nootripathu.

P-1069

Identification of Cold Regulated Genes in *Poncirus trifoliata* (L.) Ref. Using A Subtractive Library. MEHTAP SAHIN-CEVIK and Gloria A. Moore. Horticultural Sciences, University of Florida, Gainesville, FL 32611. E-mail: MSAHIN@MAIL.IFAS.UFL.EDU

Citrus is one of the most economically important fruit crops in the world, grown commercially in almost every country between latitudes 40° N and 40° S. Cultivation of citrus is mainly limited by low temperatures outside of this region. Low temperatures and freezes also result in significant damage and economic losses within the citrus growing regions. Therefore, cold hardiness is a desirable trait for introduction into commercial citrus varieties. *Poncirus trifoliata* is an interfertile *Citrus* relative that can withstand temperatures of − 26° C when cold-acclimated. To identify and characterize cold regulated genes, libraries were constructed from cold-acclimated and non-acclimated *Poncirus* seedlings using subtractive hybridization methods. A total of 192 randomly picked clones, 136 from the cold induced library and 56 from the cold repressed library, were sequenced. A number of these clones contained sequences showing homology with previously characterized cold induced as well as environmental stress regulated genes in other plants. Characterization of these genes and their roles in improving cold tolerance in citrus will be discussed.

P-1070

Improvement of Freezing Tolerance in *Brassica rapa* by Gene Transfer of a Stress-inducible Transcription Factor, DREB1A. H. TSUKAZAKI[1], Y. Kuginuki,[1] K. Hatakeyama[1], M. Fujimura[1], M. Kasuga[2], K. Yamaguchi-Shinozaki[2], T. Suzuki[1]. [1]National Institute of Vegetable and Tea Science (NIVTS) and [2]Japan International Research Center for Agricultural Sciences (JIRCAS). 360 Kusawa, Ano, Mie 514–2392, Japan. E-mail: tsuka@affrc.go.jp

Cabbage and Chinese cabbage are representative open cultured vegetables in the world. The damage by freezing or frost in the winter is one of barriers to supply them stably for the years. To improve stress-tolerance of these plants by gene transfer we tried to use a gene for stress responsive transcription factor, *DREB1A/CBF3*. The transcription factor DREB1A specifically interacts with the dehydration responsive element (DRE/CRT) and induces expression of many genes involved in environmental stress tolerance in *Arabidopsis*. We have shown that overexpression of the DREB1A cDNA activates the expression of stress tolerance genes and resulted in improved tolerance to drought, salt and freezing using transgenic *Arabidopsis*. To apply this system to *Brassica rapa*, *Brassica* plants were transformed with a vector expressing the DREB1A cDNA driven by the 35S CaMV promoter. We analyzed the transformants and found that *DREB1A* was overexpressed in the selfed progeny of transformants. It is known that overexpression of *CBF3/DREB1A* elevate the expression of a gene for the key enzyme for proline (Pro) biosynthesis, P5CS and increase the Pro levels in the transgenic *Arabidopsis*. We examined Pro levels in leaves of the transformants overexpressing the *DREB1A* transgene. In non-cold acclimated transformants, Pro was accumulated 3- to 43-folds higher than those of control plants (non-transformants and GUS transformants) and some plants were higher than cold-acclimated (5° C for 2 weeks) control plants. The Pro levels in the transformants overexpressing DREB1A increased further by cold acclimation and was 2.6- to 5.1-folds higher than those of control plants. At present we carry out stress resistant tests to confirm the freezing tolerance in transformants.

P-1071

Expression of Vitreoscilla Hemoglobin Enhances Growth of *Hyoscyamus muticus* Hairy Root Cultures. A. W. WILHELMSON, P. T. Kallio, K.-M. Oksman-Caldentey, and A. M. Nuutila. VTT Biotechnology, FIN-02044 VTT, Espoo, Finland. Institute of Biotechnology, ETH-Zürich, Switzerland. E-mail: annika.wilhelmson@vtt.fi

Expression of the *vhb* gene encoding the hemoglobin from *Vitreoscilla* sp. has been shown to improve growth properties and productivity of various microorganisms, plants, and mammalian cells. In *Vitreoscilla*, VHB expression is up-regulated by hypoxic conditions, and therefore its heterologous expression has been used to alleviate physiologically unfavorable effects of hypoxia. The *vhb* gene was introduced into *Hyoscyamus muticus* (Egyptian henbane), which was chosen for its capability of producing high amounts of tropane alkaloids. Hairy roots were obtained by transformation with *Agrobacterium rhizogenes*. The transformed roots were cultured on modified solid B5 medium and transferred to liquid medium for growth analysis. The hairy roots expressing *Vitreoscilla* hemoglobin showed some phenotypic variation and were in general lighter in color than the controls. The transgenic hairy roots showed improved growth in modified liquid B5 medium.

P-1072

Cloning Lemma- and Palea-specific Promoters in Barley. T. Abebe[1], M. L. Federico[1], J. Fu[1], R. Skadsen[2], and H.F. Kaeppler[1]. [1]Department of Agronomy, University of Wisconsin, Madison, WI 53706 and [2]USDA/ARS/CCRU Madison, WI 53705. E-mail: tabebe@facstaff.wisc.edu

Tissue-specific promoters are required for directing expression of economically important genes in target tissues. We are interested in developing lemma- and palea-specific promoters for expressing antifungal genes in barley spikes. The lemma and palea of cereals are sterile bracts that nourish florets and developing kernels. They also protect florets and kernels from mechanical damage and pathogen infection. Because of their unique functions, it is expected that specific genes are preferentially expressed in these organs. These genes are potential sources of tissue-specific promoters. We have constructed cDNA libraries from three developmental stages of the lemma and palea (elongating, gelatinous and early dough kernel stages) of the barley (Hordeum vulgare L.) cultivar Morex using the suppression subtractive hybridization (SSH) method. Using the pooled lemma/palea cDNA as a tester and the flag leaf as a driver, 300 cDNA clones were obtained. Differential screening and northern analysis of selected clones showed that the cDNAs are preferentially expressed in the lemma and palea compared to the leaf. A similarity search to the GenBank databases identified genes with known and unknown functions. A limited number of novel genes were also identified. Among the genes with known functions were those involved in photosynthesis, reactive oxygen species (ROS) scavenging, cell wall biosynthesis, microtubule formation and metabolism. Differential expression of these genes is consistent with the proposed role of the lemma and palea in providing carbohydrates and protection for the florets and developing seeds. Currently we are cloning the promoters of selected lemma- and palea-specific clones by PCR and testing them by particle bombardment.

P-1073

Sequence Analysis of the T-DNA: Barley Genomic DNA Junctions After *Agrobacterium*-mediated Barley (*Hordeum vulgare* L.) Transformation. FREDY ALTPETER[1*], Yu-Da Fang[2], Chakradhar Akula[2]. [1]University of Florida, Agronomy Department, Laboratory of Molecular Plant Physiology, 2191 McCarty Hall, P.O. Box 110300, Gainesville FL 32611–0300 and [2]Institut für Pflanzengenetik und Kulturpfanzenforschung Gatersleben, AG Gentransfer, Corrensstrasse 3, 06466 Gatersleben, Germany. Email: faltpeter@mail.ifas.ufl.edu

Agrobacterium-mediated barley transformation promises many advantages compared to alternative gene transfer methods. Using green fluorescent protein (GFP) as a non-destructive visual marker allowed us to identify single-cell recipients of T-DNA at an early stage, track their fate and evaluate factors that affect T-DNA delivery. Southern blot- and progeny segregation analysis revealed a single copy T-DNA insert in more than half of the transgenic barley plants. Results of sequence analysis of the T-DNA/barley genomic DNA junctions will be discussed.

P-1074

Non-destructive Monitoring of Expansin Promoter Activity During Germination of Transgenic *Arabidopsis* Seeds. KENT J. BRADFORD and Feng Chen. Department of Vegetable Crops, One Shields Avenue, University of California, Davis, CA 95616–8631. E-mail: KJBRADFORD@UCDAVIS.EDU

Expansins are plant cell wall proteins thought to mediate cell expansion and cell wall modification. Among three expansin genes isolated from germinating tomato seeds, expression of one of these (*LeEXP8*) was localized by tissue printing to the cortical tissue of the elongation zone of the radicle and was correlated with completion of germination under various conditions. To develop a non-destructive reporter to monitor promoter activity in individual germinating seeds, the promoter region of the *LeEXP8* gene was fused with GUS and LUC reporters and transformed into *Arabidopsis thaliana*. GUS staining showed that as in tomato, promoter activity was mainly in root tissue of transgenic seeds and seedlings. Seeds homozygous for a single pLeEXP8::LUC insert were used to assay *LeEXP8* promoter activity in individual seeds imbibed under various conditions. Individual seeds were monitored repeatedly over time for both LUC expression and germination. Although dormancy, low water potential or ABA affected germination time courses similarly, *LeEXP8* promoter expression exhibited distinct patterns under each condition, suggesting different modes of gene regulation. These results demonstrate the feasibility of non-destructive monitoring of specific promoter activity in individual seeds during germination.

P-1075

Identification, Isolation, and Characterisation of Stage- and Tissue-specific Promoters for Expression of Genes in Cereals. S. BROEDERS[1], L. Altschmied[2], E. Grützemann[1], F. Altpeter[3], and J. Kumlehn[1]. [1]Institut für Pflanzengenetik und Kulturpflanzenforschung, Department of Molecular Cell Biology, Gene Transfer Group, Corrensstrasse 3, 06466 Gatersleben, Germany; [2]Institut für Pflanzengenetik und Kulturpflanzenforschung, Department of Molecular Genetics, Expression Mapping Group, Corrensstrasse 3, 06466 Gatersleben, Germany; and [3]University of Florida, Institute for Food and Agricultural Sciences, Agronomy Unit, 2191 Mc Carty Hall, PO Box 110300, Gainesville, FL 32611–0300. E-mail: BROEDERS@IPK-GATERSLEBEN.DE

The aim of the project is to identify, isolate and characterize promoters that conduct expression during a certain stage of the plant development or in a specific tissue. For the identification of genes with the desired tissue- and stage-specific expression, cDNA-arrays were established. All analysed clones came from 5 different cDNA libraries made from etiolated leaves, roots of seedlings, developing seeds, leaf epidermis and roots under starvation. Subsequently, BAC clones that carry the candidate genes were identified and sequenced. T-DNA vectors, containing either the beta-glucuronidase gene (*gus*) or the green fluorescent protein gene (*gfp*) as a reporter gene, were constructed. During the amplification of these genes, unique restriction sites were introduced at their 5end. In this way the promoters, isolated from the BAC clones by PCR, could easily be introduced upstream of these reporter genes. As selectable markers, NPTII and HPT-1 were chosen for wheat and barley, respectively. To test the functionality of the constructs, the constitutive promoter of the rice actin 1 gene was introduced and the vectors were transformed to the crops. Since both GUS and GFP expression was observed in transient assays, we concluded that the constructs are useful to characterize other promoters. Preliminary results of the analysis of tissue- and stage-specific promoters will be presented.

P-1076

Evaluation in Cauliflower of Genetic Elements of tCUP, a Novel Constitutive Cryptic Promoter. ETSUKO TSANG[1], D. Brown[1], B. Miki[2], T. Ouellet[2], K. Wu[2], L. Tian[1], and L. Kott[3]. [1]Agriculture and Agri-Food Canada, Southern Crop Protection and Food Research Centre, London, Ontario, N5V 4T3; [2]Agriculture and Agri-Food Canada, Eastern Cereal and Oilseed Research Centre, Ottawa, Ontario, K1A 0C6; and [3]University of Guelph, Department of Plant Agriculture, Guelph, Ontario, N1G 2W1; E-mail: browndc@em.agr.ca

A cryptic, constitutive, TATA-less promoter known as tCUP (**t**obacco **c**onstit**u**tive **p**romoter), isolated from tobacco by T-DNA tagging, is capable of activating gene expression in a wide variety of plants. The core promoter region, upstream region and untranslated mRNA leader region of tCUP were evaluated in a (cauliflower) *Brassica oleracea L.* var *botrytis* test system. Enhancer elements were detected in the upstream region, the core promoter region was defined, and the mRNA leader sequence was found to be critical for maintaining high levels of gene expression. The addition of various consensus TATA box sequences resulted in significant increases in gene expression. Putative cryptic *Inr* sites and an important domain in the +30 to +40 region (possibly a plant DPE element) were discovered. The results show that cryptic regulatory elements are structurally and functionally similar to regulatory elements associated with expressed genes. The results give insight into the nature of the core promoter elements and demonstrate that the tCUP gene expression system may be an effective

P-1077

The Effect of Cold-induced Dormancy on Post-transcriptional Gene Silencing in Plum Clones Containing the Plum Pox Potyvirus Coat Protein. ANN CALLAHAN, Ralph Scorza, Laurene Levy, Vern Damsteegt, and Michel Ravelonandro. Appalachian Fruit Research Station, USDA-ARS, Kearneysville, WV 25430; National Plant Germplasm Quarantine Center, USDA-APHIS-PPQ, Beltsville, MD; Foreign Disease-Weed Science Research Unit, USDA-ARS, Ft. Detrick, MD; IBVM-INRA, Bordeaux, France. E-mail: ACALLAHA@AFRS.ARS.USDA.GOV

Plum trees containing the coat protein gene (CP) from plum pox potyvirus (PPV) were inoculated with PPV through graft inoculation in a containment greenhouse. Only one line, C5, was highly resistant to infection by PPV. The mechanism of resistance appears to be post-transcriptional gene silencing (PTGS). In herbaceous systems, PTGS has been shown to be 're-set' following seed germination. It is important to know in a perennial fruit tree, if PTGS can be maintained rather than 're-set', through the yearly cycles of growth and dormancy. Leaf samples were collected from several clones of PPV- CP transformed plums over a period of three years before and after cold-induced dormancy (CID) periods that simulated winter dormancy and spring growth. The levels of viral RNA and transgene CP RNA were measured and compared in susceptible PPV-CP transgenic clones and the resistant C5 clone prior to CID, one month after CID (first leaf expansion) and three months post-CID. There was at least tenfold more viral RNA and two-fivefold more transgene RNA detected in the susceptible clones than in C5 as would be expected since the PPV-CP transgene is silenced in C5. The levels of both transgene and virus increased following CID in both the susceptible lines and C5, although the increases in C5 was significantly lower than in the susceptible clones. Either a re-setting of PTGS (in C5), or the effects of release from dormancy (in all clones) allowed more virus to be detected and a higher level of transgene accumulation immediately post-CID. Our results indicate an environmental influence on PTGS in a temperate woody perennial. This phenomenon has implications for the deployment PTGS-mediated resistance in the field.

P-1078

Expression of Putative Antifungal and Resistant Cascade Regulatory Genes in Oat and Their Effects on Fungal Resistance. ALVAR CARLSON[1], Heidi Kaeppler[1], and Ron Skadsen[2]. [1]Department of Agronomy, 1575 Linden Dr., Madison WI 53706 and [2]USDA ARS Cereal Crops Research Unit, 501 Walnut St., Madison WI 53705. E-mail: ARCARLSON@STUDENTS.WISC.EDU

Cultivated oat (*Avena sativa* L.) is an agronomically important cereal crop both locally and globally. Oat production is significantly reduced in many parts of the world, including North America, due to fungal infection, including the main disease, crown rust (*Puccinia coronata*). There are considerable efforts to breed for fungal resistance in current oat breeding programs, but evolving virulence in the pathogens requires a more detailed look at understanding the endogenous resistance pathways and developing alternative methods towards solutions for improved resistance. Seventy-nine independent transgenic oat lines were produced, each containing one or two of four putative antifungal genes. The first two transgenes, hordothionin and permatin, are PR (pathogenesis-related) genes. Both the barley (*Hordeum vulgare* L.) hordothionin gene and the oat permatin gene are thought to act via pore formation or disruption of the fungal plasma membrane. These genes are induced during plant disease response and we hypothesize their constitutive expression will increase oat's fungal resistance. The second two transgenes, *Arabidopsis* NPR1 and the rice NPR1 homolog, NH1, function in the cascade signal pathway that induces the expression of PR genes during pathogen attack. NPR1 has already been shown to increase resistance in heterologous species. Pure breeding transgenic and null lines have been advanced for each event, and results from seedling-based resistance assays, fungal spore germination on plant extracts, and seed germination on fungal cultures will be presented.

p-1079

Cloning of Genes that Encode Transcription Factors that Bind to the Chi2;1 Promoter of Tomato Using the Yeast One-hybrid System. PICK KUEN CHAN, A. R. Siti Suhaila, C. W. Choong, O. Yasmin, and K. Harikrishna. Department of Biotechnology, Faculty of Food Science and Biotechnology, Universiti Putra Malaysia, UPM Serdang 43400, Malaysia. E-mail: pickuen@hotmail.com

The ability to regulate the expression of genes is fundamental to most biological phenomena such as development, differentiation, cell growth, and responses to environmental signals. Transcriptional regulation of gene expression relies on the recognition of *cis*-acting elements by sequence specific DNA-binding of the corresponding gene/s. To understand the interaction that occurs between *cis*- and *trans*-regulatory elements, a promoter region from a tomato floral stylar endochitinase, Chi2;1 was used to facilitate this study. The yeast one-hybrid system was used to isolate transcription factors that recognize elements within the Chi2;1 promoter. Approximately 5.95×10^6 yeast transformants were screened using six yeast reporter strains carrying regions of the Chi2;1 promoter. Thirty-eight putative positive clones encoding transcription factors were identified and isolated based on a positive b-galactosidase assay. The DNA sequence of these clones was determined and compared to known DNA sequences in the GenBank database. DNA mobility shift assays are being carried out to confirm the DNA binding specificity of these clones to the Chi2;1 promoter. Further characterization of these putative clones by Northern blotting and *in situ* hybridization is underway. It is hoped that information obtained will lead to a better understanding of the regulation of style specific expression in tomato.

P-1080

Expression of 2S Albumin Gene in Developing Seeds of *Ricinus communis*. Grace Q. Chen, Xiaohua He, Lucy Liao, Thomas A. McKeon. USDA-ARS West Regional Research Center, Albany, CA 94710.

One limitation on cultivation of the castor plant is the presence of highly allergenic components in the castor seed. Safe handling of the castor meal resulting from oil extraction requires heat inactivation of the allergen. As part of a genetic approach to eliminating the allergenic components from castor, we have conducted a genetic analysis of the gene family. In accordance with previous results for the Ric c1 and Ric c3 coding regions for 2S albumins, the primary allergenic component of castor, we have found that there is a family of at least 3 genes. Based on gene sequences from 2 castor cultivars and 9 expressed sequences, the 2S albumins produced during castor seed development are highly conserved. This conservation of what is apparently a storage protein suggests that only one gene is expressed during development, or that it plays a role requiring maintenance of the identical amino acid sequence. Northern analysis indicates that the 2S albumin gene is highly expressed in the endosperm tissue of ripening castor bean seed.

P-1081

Late-flowering Transgenic Radish Produced by the Cosuppression of GIGANTEA (GI) Gene. *I. S. CURTIS, H. G. Nam, J. Y. Yun, K. H. Seo, and *Y. Kamiya. Laboratory of Plant Molecular Genetics, POSTECH, Pohang, 790–784, Republic of Korea; *Plant Science Center, RIKEN, Saitama, 351–0198, Japan. E-mail: iscurtis@postman.riken.go.jp

Radish is a long-day, highly nutritional and medicinally important crop cultivated widely in S/E Asia. Korean genotypes are cold-sensitive and so bolt during the autumn season, resulting in dramatic losses in crop yield. *GIGANTEA* (*GI*) gene is important in regulating photoperiodic flowering and controlling circadian rhythms; *gi* mutants of *Arabidopsis* exhibit delayed flowering under long-days. Recent developments in the gene manipulation of radish by floral-dip, has enabled the commercially important genotype 'Jin Ju Dae Pyong' to be genetically modified by *Agrobacterium tumefaciens* strain AGL1. Twenty-five plants were dipped into a suspension of *Agrobacterium* carrying pCAMBIA3301 with 2.5 kb antisense *GI* gene fragment from *Arabidopsis*, along with *gus*A and *bar* reporter genes, all under the control of a CaMV 35S promoter. From a total of 1462 seeds harvested from floral-dipped plants, 16 Basta-resistant T1 plants were found to have GUS activity (1.1% transformation efficiency). Southern analysis confirmed the integration of one or two copies of the *gus*A gene in these herbicide-resistant plants. Northern blots revealed that the level of *GI* transcript was inversely proportional to the time of bolting and flowering in transgenic plants. In the progenies of eleven, randomly selected, T1 plants (T2 generation), bolting and flowering times were delayed by a maximum of 17 and 18 days respectively, compared to wildtype; 23 and 26 days delayed compared to pCAMBIA3301 transformants (positive control), respectively. This early study suggests that cosuppression of the *GI* gene could possibly extend the growing season of radish into the fall period and so benefit the famine-stricken countries that rely on the crop as part of their daily diet.

P-1082

Enhancement of Wheat Grain Yields by Delaying Leaf Senescence. SASHA DASKALOVA*, A. Sorokin*, N. W. Scott*, A. Slater*, M. R. Fowler*, M. Kaminek**, A. Gaudinova**, M. Trckova**, and M. C. Elliott*. *The Norman Borlaug Institute, De Montfort University, Scraptoft, Leicester LE7 9SU, UK. **Institute of Experimental Botany, Academy of Sciences of the Czech Republic, Rozvojova 135, 16502 Praha 6, Czech Republic. E-mail: SDaskalova@dmu.ac.uk

The effect of extending the period of photosynthesis on final grain yield in transgenic wheat has been investigated. The *Agrobacterium ipt* gene was placed under the control of the *Arabidopsis thaliana SAG12* promoter and introduced to immature embryos by bombardment. Leaf senescence and the onset of anthesis were delayed by about two weeks in the transgenic plants that were produced. The T_0 progeny showed a low level of fertility but the fertility was restored in the T_1 generation. The *ipt*-positive T_1 plants showed an increase in grain weight ranging from 5% to 30%. Plants from one line, C8, which exhibited an increase in grain number and the greatest increase in grain weight, have been studied in detail. In these plants the accumulation of starch and storage proteins in the seeds was significantly enhanced, total starch and protein contents were 30% and 35% higher respectively, than control plants. The observed changes correlate with an increase in cytokinin levels.

P-1083

Gene Targeting in *Arabidopsis* after *In Planta Agrobacterium*-mediated Transformation M. ENDO[1,2], K. Osakabe[2,3], Y. Nomura[2,3], H. Ichikawa[2], S. Nishimura[1], S. Toki[2]. 1. Tsukuba U, 2. NIAS, and 3. BRAIN. E-mail: mendo@affrc.go.jp

The recent study of moss indicates there might be a close correlation between the haploid state and efficient homologous recombination in higher plants. A single amino-acid change (S653N) in the acetolactate synthase (ALS) protein of *Arabidopsis* confers resistance to the herbicide imazapyr. We have constructed a binary vector which has a deleted, non-functional fragment from acetolactate synthase gene, carrying the mutant site specifying herbicide imazapyr resistance. After *in planta Agrobacterium*-mediated transformation of *Arabidopsis*, we have selected imazapyr resistant plants. Molecular analyses of these plants will be presented.

P-1084

Gene Profiling in Oil Palm Tissue Culture Through the Generation of Expressed Sequence Tags (ESTs) and Microarray Analysis. E. T. L. LOW, A. Halimah, C. Y. T. Amos, M. S. Elyana, S. Rajinder, and S. C. Cheah. Advanced Biotechnology and Breeding Centre, Malaysian Palm Oil Board (MPOB), PO Box 10620, 50720 Kuala Lumpur, Malaysia. Email: lowengti@mpob.gov.my

Among the world's major oil crops, the oil palm has the highest oil yield per unit-cultivated area. Palm oil from Malaysia accounts for close to 60% of the world's total palm oil exports. The high demand for palm oil requires a constant improvement of the oil palm trees. Conventional methods of breeding for oil palm is time consuming. The long breeding cycle (10–12 years) and a wide range of variation in oil yield per palm have made tissue culture of oil palm an attractive prospect for producing uniform plantlets from selected high yielding palms. A comprehensive study of genes expressed during oil palm tissue culture is presently being carried out, through the generation of ESTs and the use of DNA microarray technology. ESTs generated provide an overall picture of the genes involved in tissue culture and forms the framework for large-scale functional analysis of thousands of genes. ESTs generated from oil palm cDNA libraries are used as probes on an oil palm DNA chip (microarray). The oil palm chip is then used to bridge the gap between sequence information and functional genomics and thus provide information on genome-scale sampling of gene expression patterns. At present, 4,189 clones from the embryoid cDNA libraries and 2,981 clones from the callus cDNA libraries have been sequenced. A subset of these genes was spotted on the oil palm chip. Initial observations have shown differential gene expression between callus and leaf (explant) samples.

P-1085

Cloning and Promoter Analysis of a Barley Gene Encoding a Lipid Transfer Protein. M. L. FEDERICO[1], R. Skadsen[2] and H. F. Kaeppler[1]. [1]Department of Agronomy, University of Wisconsin, Madison, WI 53706 and [2]USDA/ARS, Cereal Crops Unit, Madison, WI 53705. Email: MLFEDERICO@STUDENTS.WISC.EDU

Fusarium head blight, or scab, is a fungal disease that causes significant yield and quality reductions in barley and wheat worldwide. Sources of immunity have not been identified, limiting breeding efforts for enhanced resistance. Genetic transformation, therefore, represents a promising means for controlling this disease. Currently, constitutive promoters are chosen to drive expression and test the efficacy of candidate antifungal proteins against *Fusarium graminearum*. Ideally, however, expression of antifungal genes should be restricted to the spike tissues where infection takes place, resulting in stopping the fungus before it spreads and colonizes the endosperm. With that goal in mind, we cloned and characterized a novel barley gene, *EpiLTP*, which is highly expressed in the epicarp, one of the first spike tissues to be colonized by *F. graminearum*. Several putative *cis*-acting DNA elements, which are responsive during plant development and environmental stimuli, were found in the promoter and 5'UTR regions of *EpiLTP*. Northern blot analyses revealed that *EpiLTP* is also highly expressed in the coleoptile and embryo, but not in endosperm, anther, rachis, awn, leaf, stem or root tissues. The promoter region, 5'UTR, and the sequence encoding a putative signal peptide were cloned using PCR-based methods. A series of 5' and 3' promoter deletions were studied in transient expression assays. A minimal construct containing 244 bp of promoter and the 5' UTR (-244/+84) was sufficient to drive preferential expression in the epicarp, coleoptile and embryo tissues. Stable transformation of barley cv. Golden Promise with a *EpiLTP-GFP* promoter fusion is underway in order to study the regulation and tissue-specificity of this novel barley gene.

P-1086

Development of an Automated Image Collection System for Generating Time-lapse Animations of Plant Tissue Growth and Green Fluorescent Protein Gene Expression. M.T. Buenrostro-Nava and J.J. FINER. OARDC/The Ohio State University, Wooster, OH 44691. Email: finer.1@osu.edu

Over the past few years, robotics and image analysis has transformed many different fields, from manufacturing industries to high-throughput genomics efforts. If precision, speed and consistency are needed for routine and repeatable manipulations, robotics should be considered. Our goal was to develop an automated system to quantitatively evaluate and track both tissue growth and gene expression over time using the green fluorescent protein (GFP) as a reporter gene. The automated system consisted of a belt-driven XY positioning table and a CCD camera mounted on a dissecting microscope. The platform was driven by two dual stepper motors, which advanced the table in either the X or Y direction. After each sample was positioned under the microscope and the image was acquired and stored, the platform was directed to the position reference and then to the next position; this process was repeated for each of the samples on the platform. To precisely control both the XY table and the CCD camera, a C base code for the MD2 software, provided with the XY table, was modified using the Visual C++ 6.0 compiler. To control the camera and to obtain sequential images, new applications were written using a dynamic link library (DLL) file and header files. The modified algorithms were integrated into a single program to obtain a precise coordination between the XY table and the camera. Digital images were acquired, stored, processed and analyzed. To maintain the specimens under aseptic conditions, the automated system was placed in a horizontal flow laminar hood located in a culture room with controlled environmental conditions. This system has been used to quantify gfp gene expression in transiently and stably transformed tissues and to generate time-lapse animations of tissue growth and gfp gene expression in those tissues.

P-1087

Agrobacterium-mediated Transformation of Maize Embryos Using a Standard Binary Vector System. B. FRAME, H. Shou, R. Chikwamba, Z. Zhang, C. Xiang, T. Fonger, S. E. Pegg, B. Li, K. Wang. Plant Transformation Facility, Iowa State University, Ames, IA 50011. E-mail: bframe@iastate.edu

We report routine production of stable, fertile transgenic maize using an *Agrobacterium tumefaciens* standard (non-super) binary vector system. Hi II immature zygotic embryos were infected with *A. tumefaciens* strain EHA101 containing a CaMV 35S-*gus*-intron and CaMV 35S *bar* cassette and co-cultivated in the presence or absence of 400 mg/L L-cysteine. The cysteine treatment led to an improvement in the amount and distribution of transient GUS expression observed in cells targeted for transformation but resulted in a reduction in the proportion of embryos giving rise to embryogenic callus. Rate of stable transformation (number of bialaphos reistant events recovered per 100 embryos infected) was significantly higher from embryos co-cultivated in the presence of 400 mg/L cysteine than from those co-cultivated in the absence of cysteine. Integration, expression and inheritance of the *bar* and *gus* transgenes in R_0, R_1 or R_2 plants were confirmed by Southern blot and progency analyses. To date we have achieved an average stable transformation efficiency of 5.5% using this *Agrobacterium* standard binary vector to transform Hi II immature zygotic embryos. We are working to improve transformation efficiency by optimizing cysteine concentration in co-cultivation medium while using this protocol to compare stable transformation efficiencies achieved with other standard binary vectors.

P-1088

Microalgae in Biotechnology. M. FUHRMANN, A. Eichler-Stahlberg, and P. Hegemann. Department of Biochemistry, University of Regensburg, 93053 Regensburg, Germany. E-mail: markus.fuhrmann@vkl.uni-regensburg.de

The use of algae in biotechnology is by now limited to fast biomass production and to the isolation of cellular compounds, e.g vitamins. Algal cultures are cheap, easily grown in large amounts in pure mineral medium under sterile conditions, and are supposed to be free of human pathogens. Recent progress in transgenic technology allows a further exploitation of different green and diatom algae as new expression systems for both pharmaceutical and technological purposes. Especially the use of synthetic genes and the development of efficient promoters improved transgenic technology in algae during the last years. Transformation can be carried out without any antibiotic selection markers simply with linear DNA fragments, complementing selected metabolic mutations. The presentation will include recent results in model protein expression, taking into account like yield, purity and functionality of the produced proteins. The further application in the production of pharmaceutical model proteins will be discussed.

P-1089

Understanding and Controlling the Wheat and Barley Transformation Process. WENDY A. HARWOOD, Valerie Bourdon, Shona Ross, Lorelei Bilham, Judith Harden, Allan Wickham, David Lonsdale, John W. Snape. Crop Genetics Department, John Innes Centre, Norwich Research Park, Colney, Norwich NR4 7UH, UK. E-mail: wendy.harwood@bbsrc.ac.uk

Wheat and barley transformation is now routine, at least for certain cultivars. The challenge now is to acquire a more detailed understanding of the transformation event, to be able to control transgene expression and to determine whether the transformation process has any unexpected consequences for the plant and therefore for foodstuffs made from it. To address these issues we have firstly examined the transgene integration site in detail. As well as molecular analysis, we have determined the physical and genetic location of the transgenes and sequenced the regions flanking them in transgenic barley. Transgene expression has been examined by following expression of the firefly luciferase gene over five generations in selected wheat and barley lines. This study has shown large differences in expression levels between transgenic lines and between different generations of particular lines. The effect, on transgene expression, of adding additional introns to the luciferase coding region has been studied. Additional introns had a significant effect on transgene expression levels both within and between generations in wheat. However, addition of MARs sequences did not influence expression stability. A number of methodologies have been considered for the screening of transgenic plants for unexpected consequences of the transgene insertion. Initial studies of transgenic barley lines using NMR suggest that this may provide a suitable method for detecting unexpected changes in metabolite profiles due to the transformation process.

P-1090

Transient GUS Gene Expression in Transgenic Lentil (*Lens culinaris* Medik) Lines from Syria. F. HASSAN, H. Kiesecker, and H. J. Jacobsen. LG Molecular Genetic, Hannover University. Herrenhauser str.2–30419 Hannover, Germany. E-mail: hassan@lgm.uni-hannover.de http://www.lgm.uni-hannover.de

The overall goal of this work is to develop an efficient transformation system for four lines of lentil (*Lens culinaris* Medik) obtained from the International Center for Agricultural Research in the Dry Areas (ICARDA), Aleppo, Syria. These are: ILL6994, ILL5883, ILL7201, and ILL7012. Agrobacterium strains EHA101 and LBA4404 containing the plasmid binary vector pIBGUS were used for transformation of all 4 lines using decapiteted embryos and half embryo attached cotyledons as explants. Selection was carried out with 50 mg/l Kanamycine and 40 mg/l Gentamycine. Transformation was confirmed in our primary transgenics by a GUS assay for the expression of the GUS gene. Presence of the GUS gene was demonstrated by transient gene expression assay using the histochemical GUS staining procedure (Jeffersson, 1987). Transient GUS gene expression was evaluated three days after co-culture. Significant differences in GUS expression were observed with respect to the Agrobacterium strain used and explant source.

P-1091

Improvement of *Agrobacterium*-mediated Transformation and Delivery of Large Intact DNA Fragments into Barley (*Hordeum vulgare* L.) as a Tool for Efficient Positional Cloning and Pathway Engineering. G. HENSEL[1], R. Vishnoi[1], F. Altpeter[2], and J. Kumlehn[1]. [1]Institut für Pflanzengenetik und Kulturpflanzenforschung (IPK), AG Gentransfer, Corrensstr. 3, 06466 Gatersleben, Germany and [2]Present address: University of Florida, Agronomy Department, Laboratory of Molecular Plant Physiology, 2191 McCarty Hall, P.O. Box 110300, Gainesville FL 32611–0300. E-mail: hensel@ipk-gatersleben.de.

Accelerated positional cloning and pathway engineering have a high potential for genome research and barley improvement. Therefore an efficient method for the transfer of foreign DNA is necessary. *Agrobacterium*-mediated genetic transformation of barley was adapted due to the advantages of preferred integration in transcribed regions, simple integration pattern and high potential for elemination of marker genes through segregation upon co-transformation. Initially, the work focussed on the development of a highly efficient in vitro regeneration system for barley and selection of suitable genotypes for genetic manipulation. Based upon the established method for multiple shoot formation from immature embryos, the transformation with vectors harbouring different selectable markers (*hph, hpt*) or reporter genes (*gfp, uid*A) was done and the resulting observations will be presented. To improve map-based cloning strategies via *Agrobacterium*-mediated transformation of barley, the transfer of large DNA fragments up to intact BAC is desirable. The limiting fragment size of common vectors appears to be about 30 to 40 kb. New binary BIBAC (binary bacterial artificial chromosome) and TAC (transformation-competent artificial chromosome) vectors were recently created and the integration of a 150 kb human DNA fragment into tobacco and an 80 kb fragment into Arabidopsis was shown, respectively. A set of vectors with different fragment sizes have been designed and following *Agrobacterium*-mediated transformation of barley, the inserted intact fragment sizes, the frequency of the transfer events and the individual integration pattern will be investigated.

P-1092

High Throughput Production of Transgenic *Physcomitrella* Plants. A. HOHE, J. Granado, G. Schween, R. Reski. Plant Biotechnology, Freiburg University, Sonnenstraße 5, D 79104 Freiburg, Germany. E-mail: hohe@uni-freiburg.de Website: www.plant-biotech.net

The moss *Physcomitrella patens* is of growing interest as a model organism in plant functional genomics, as it is the only plant showing high rates of homologous recombination in its nuclear DNA, which allows targeted knockout of genes. As a basis for analysis of gene functions and isolation of novel genes a saturated mutant collection of transgenic Physcomitrella plants is currently produced by transformation with transposon mutagenized cDNA libraries of Physcomitrella. This requires a high throughput transformation system, which was developed based on the PEG mediated transformation protocol widely used for Physcomitrella. Semicontinuous photoautotrophic bioreactor cultures of Physcomitrella in modified Knop medium were established and the transformation protocol optimised regarding the plant material for protoplast isolation, DNA conformation and amount as well as several parameters of the transformation procedure. As it was expected to produce also metabolic mutants, a full medium was developed that was supplemented with several organic compounds in order to rescue auxotrophic mutants. Using this system currently 800 transgenic Physcomitrella plants are produced per week. Quality control of the first 23,400 transformants on ploidy level and stable integration of the transgene revealed that 91.2% of the plants were haploid and 97.6% were stable transformants. This work has been performed in a joint project with BASF Plant Science GmbH.

P-1093

Utilization of Barley Seeds as Bioreactors: Analyzis of Developmentally and Spatially Regulated Promoters. H. HOLKERI, K.-M. Oksman-Caldentey, and A. M. Nuutila. VTT Biotechnology, FIN-02044 VTT, Espoo, Finland. E-mail: heidi.holkeri@vtt.fi

Cereal grain expression may provide an economical mean for large-scale production of therapeuticals and feed enzymes in the future. Efficient utilization of cereal grains as bioreactors, however, requires a system for regulating the transgene expression developmentally and spatially within the endosperms of cereal seeds. Malting barley has been traditionally bred for high enzyme production capabilities during the seed germination. The germination specific promoters of barley can therefore also be used to control expression of heterologous proteins during germination. Alternatively, promoters active during seed maturation can be utilized to produce heterologous proteins during grain filling. To develop an optimal system for efficient and economical transgene expression in barley grain, we are currently testing the efficiencies of promoters of several barley storage protein genes. The activities of both germination specific, as well as maturation specific promoters will be compared using heterologous genes coding for GUS and GFP as reporters. The reporter constructs will be expressed transiently in barley endosperms and the results obtained will be utilized to create new barley varieties stably expressing desired transgenes in the seed endosperm with peak expression at defined times.

P-1094

A Large Population of Small Chloroplasts in Tobacco Leaf Cells Allows More Effective Chloroplast Movement than a Few Enlarged Chloroplasts. W. J. JEONG, Y. I. Park, K. H. Suh, J. A. Raven, O. J. Yoo, and J. R. Liu. [1]Plant Cell Biotechnology Laboratory, Korea Research Institute of Bioscience and Biotechnology (KRIBB), Taejon 305–333, Korea; [2]Department of Biology, Chungnam National University, Taejon 305–764, Korea; [3]Department of Biology, Taegu University, Taegu 713–714, Korea; [4]Division of Environmental and Applied Biology, School of Life Science, University of Dundee, Dundee DD1 4HN, Scotland; [5]Department of Biological Sciences, Korea Advanced Institute of Science & Technology, Taejon 305–701, Korea. E-mail:jrliu@mail.kribb.re.kr

We generated transgenic tobacco plants that contained only one to three enlarged chloroplasts per leaf mesophyll cell by introducing *NtFtsZ1–2*, a cDNA for plastid division. These plants were used to investigate the advantages of having a large population of small chloroplasts rather than a few enlarged chloroplasts in a leaf mesophyll cell. Despite the similarities in photosynthetic components and ultrastructure of photosynthetic machinery between wild type and transgenic plants, the overall growth of transgenic plants under low and high light conditions was retarded. In wild type plants the chloroplasts moved toward the face position under low light and toward the profile position under high light conditions. However, chloroplast rearrangement in transgenic plants in response to light conditions was not evident. In addition, transgenic plant leaves showed greatly diminished changes in leaf transmittance values under both light conditions, indicating that chloroplast rearrangement was severely retarded. Therefore, under low light conditions the incomplete face position of the enlarged chloroplasts results in decreased absorbance of light energy. This, in turn, reduces plant growth. Under high light conditions the amount of absorbed light exceeds the photosynthetic utilization capacity due to the incomplete profile position of the enlarged chloroplasts, resulting in photo damage to the photosynthetic machinery, and decreased growth. The presence of a large number of small and/or rapidly moving chloroplasts in the cells of higher land plants permits more effective chloroplast phototaxis and, hence, allows more efficient utilization of low incident photon flux densities. The photosynthetic apparatus is, consequently, protected from damage under high incident photon flux densities.

P-1095

Molecular Characterization of Three Major Peanut Allergen Genes. I.-H. KANG[1], M. Gallo-Meagher[1], and P. Ozias-Akins[2]. [1]Agronomy Department, University of Florida, Gainesville, FL 32611–0300. [2]Department of Horticulture, University of Georgia, Tifton, GA 31793–0748. Email: ihkang@ufl.edu

Peanut (*Arachis hypogaea* L.) is a popular food due to its low cost and nutrition. However, as little as 1/2 a peanut can cause a fatal reaction for severely allergic individuals. Therefore, it is important to find a way to reduce the food allergy risks associated with peanut. We have examined the expression patterns of the three major peanut allergen genes, *arah1*, *arah2*, and *arah3*. The proteins encoded by these genes belong to the vicilin, conglutin and glycinin families of seed storage proteins, respectively. Total RNA was isolated from four seed developmental stages (1–4) of 12 different peanut genotypes. Northern blot analysis revealed that transcripts of all genes are evident at the earliest stage (1) of seed development. However, *arah1* transcripts continue to accumulate throughout development with a maximum level observed at the most mature stage (4), while *arah2* and *arah3* transcript levels appear to peak earlier in seed development. Expression patterns were similar for most genotypes, however there were exceptions that will be discussed. No transcripts of *arah1* or *arah2* could be detected in total RNA isolated from flowers, leaves or roots. However, a low level of *arah3* transcript could be observed in flower and leaf tissues. Southern blot analysis revealed a low copy number of *arah1* and *arah2*, and multiple copies of *arah3* present in the tetraploid peanut genome.

P-1096

Analysis of a Pollen Preferentially Expressed Gene from *Brassica campestris* and Its Promoter. KIM HO-IL, Park Beom-Seok, Kim Hyun-Uk, and Jin Yong-Mun. Cytogenetics Division, National Institute of Agricultural Science and Technology, Suwon 441–707. E-mail hikim@rda.go.kr

We have isolated and characterized a pollen-specific gene. BAN103, from Chinese cabbage and analyzed the activity of its promoter. BAN103 cDNA and genomic clone containing the full-length gene were seqeunced. BAN103 gene is a single copy in Chinese cabbage genome and divided into three exons by two introns.The deduced sequence of 68 amino acids shows homology with Brassica oleracea pollen coat portein and several cold induced proteins. The transcriptiion of BAN103 was restricted in anthers but not in pistils,sepals or non-reproductive tissues.Its transcription is also regulated developmentally. It was first detected after microspore releasing and increased until mature pollen. The promoter of BAN103 gene was fused with GUS gene, and transformed to Chinesse cabbage and tobacco.The GUS expression was detected pollen-specifically in transgenic tobacco plants.The pollen-specific activity of this promoter was retained within 176 bp from translation start codon. The GUS transcription and translation were not coincident in transgenic tobacco pollen. GUS transcripts appeared just after microspore release and that translation started as the pollen began to dry in mature anthers.

P-1097

Isolation and Characterization of STF1-interacting Proteins Involved in the Regulation of Seedling Development. S. H. KIM, S. Y. Shin, Y. H. Song, and J. C. Hong. Department of Biochemistry, Division of Applied Life Science, Plant Molecular Biology and Biotechnology Research Center, Gyeongsang National University, Jinju, Gyeongnam 660–701, Korea. Email: jchong@nongae.gsnu.ac.kr

Germinated seedlings display contrasting developmental patterns depending on the ambient light, photomorphogenesis in the light and skotomorphogenesis in darkness. Light signal perceived by multiple photoreceptors is transduced to downstream regulators and dictate the extent of photomorphogenic development in a quantitative manner. We have previously reported a novel bZIP factor, STF1, which carries a zinc finger motif and a bZIP domain that can heterodimerize with GBF proteins of soybean. The STF1 contains two separated domains that show high homology to domains conserved in cellulose synthase in the N-terminus and HY5 in the C-terminus. The STF1 is found to be a nuclear transcription factor without carrying a transcription activity in plant and yeast system. To understand the function of STF1 in gene expression we studied STF1-interacting proteins isolated by yeast two-hybrid screen. Three different cDNAs encoding zinc-finger proteins (SZFT1, 2, 3) were isolated. These proteins belonged to a family of GATA-like proteins involved in light controlled plant response such as *Arabidopsis* STO and CONSTANS. Transcription activity of the isolated proteins were observed in both yeast and plants. Domains involved in protein-protein interaction were further analyzed. This study suggests that photomorphogenic control requires complex molecular interactions among several different classes of transcription factors such as bZIP, SZFT1 and COP1. (This work was supported by BK21 program and the grant from CFGC of 21C Frontier Research Program)

P-1098

Two T-DNA System as the Way of Introducing Unlinked Transgenes into Plants. MASHA KONONOVA, Allan Wenck, Marina Sigareva, Rody Spivey, Cathrine Kramer, Eric Dosch, Yin-Fu Chang, and Genevieve Hansen*. Syngenta Biotechnlogy Inc., Research Triangle Park, NC 27709 and *Torrey Mesa Research Institute, Syngenta, San Diego, CA 92121 E-mail: maria.kononova@syngenta.com

Agrobacterium is capable of transferring any portion of DNA flanked by border sequences (T-region). In nature, multiple T-regions are found to occur (TR and TL). Either one or both of these may be inserted into the plant DNA. T-regions may also exist on different plasmids within the same strain (de Frammond *et al.,* (1986) Mol. Gen. Genet 202:125). *Agrobacterium* is able to transfer more than one T-DNA to a plant cell where they can be integrated as linked or independent transgenes. In addition, strains containing different T-DNA's can be mixed during infection with the same results. In the literature, various versions of this system have been analyzed with co-transformation efficiencies ranging around 50%. Linkage analyses in these papers have shown quite different results with linkage being seen in a majority of cases in some papers and rarely in others. In all of these studies, few lines were analyzed and often different two T-DNA systems were used. In order to determine if there are indeed differences in two T-DNA systems, we conducted a study using three different combinations of two T-DNA systems and have analyzed segregation ratios in all of the systems: tobacco, tomato and maize—single plasmid + two T-DNA's—two plasmids, two T-DNA's in one Agro strain—mixed Agro stains with two independent plasmids. High (85 to 87%) co-transformation frequencies were observed in tobacco and tomato when the single plasmid with two T-DNA's system was used. The Segregation rate of GOI from Marker gene in two independent T1 lines of transgenic tobacco and one line of tomato was 100% (more lines are being analyzed). When two separate binary plasmids with two T-DNAs in one Agro strain system was used the co-transformation efficiency was 35–54%- for tomato. The segregation rate of GOI from Marker gene in ten independent T1 lines of transgenic tomato was 11% (more lines are being analyzed). When separate plasmids were used within the same strain, 44–72% of transformed maize plants also had the non-selected T-DNA. When two strains were mixed, 14–30% of transformed maize plants contained the non-selected T-DNA. Segregation rates for the two T-DNAs in mixed infections in maize is 71% and 32% of T1 maize segregated when single *Agrobacterium* strain was used (5/7 and 6/19 lines respectively). Studies in these two T-DNA systems are very important because this system has great potential to provide us with a tool of segregating out unlinked genes. In addition, high co-transformation frequencies allow us to put multiple genes into plants at the same time without the difficulty of cloning and re-cloning

P-1099

Cloning of Phorate-induced Genes in Peanut. CHENGALRAYAN KUDITHIPUDI[1], J. M. Davis[2], A. Morse[2], G. E. MacDonald[1], and M. Gallo-Meagher[1]. [1]University of Florida, Agronomy Department, Gainesville, FL 32611–0300 and [2]University of Florida, School of Forest Resources and Conservation, Gainesville, FL 32611–0410. Email: chengal@ufl.edu

Tomato spotted wilt virus (TSWV) is thrips vectored, occurs worldwide and causes serious losses in the field. Spotted wilt caused by TSWV, affects at least 166 plant species, including tomato, pepper, peanut, and many ornamentals. Since 1985, it has become one of the most serious and complex disease problems in the production of peanut (*Arachis hypogaea* L.) and other crops in the southeastern United States. Phorate (Thimet 20G) has demonstrated consistent, low-level suppression of TSWV above other important insecticides like imidacloprid and triamethoxam. The mechanism of TSWV suppression by phorate is unknown, but the level of thrips control obtained with phorate is **not** greater than that obtained with other insecticides. Therefore, it is unlikely that TSWV suppression is due to a decrease in vector population. Close observation in the field revealed phorate burns on the leaves of peanut. We hypothesize that phorate may induce a defense response in the peanut plant that allows it to better resist TSWV. To understand the mechanism of how phorate suppresses TSWV, we have used differential display of mRNA to identify gene products that are regulated by phorate treatment in peanut. Fifty-three differentially expressed genes were cloned and sequenced. Putative identification of these cDNAs by comparison to known sequence data has allowed us to infer some of the biochemical and molecular processes that are altered in peanut's response to phorate.

P-1100

Membrane Localization of *Arabidopsis* acyl-CoA Binding Protein ACBP2. H.-Y. LI and M.-L. Chye. Department of Botany, The University of Hong Kong, Hong Kong, China. E-mail: LIHY@HKUCC.HKU.HK

Cytosolic acyl-CoA binding proteins bind long-chain acyl-CoAs and act as intracellular acyl-CoA transporters and pool formers. Recently, we have characterized *Arabidopsis thaliana* cDNAs encoding novel forms of ACBP, designated ACBP1 and ACBP2, that contain a hydrophobic domain at the N-terminus and show conservation at the acyl-CoA binding domain to cytosolic ACBPs. We have previously demonstrated that ACBP1 is membrane-associated in *Arabidopsis*. Western blot analysis using ACBP2-specific antibodies on *A. thaliana* protein show that ACBP2 is located in the microsome-containing membrane fraction and in the subcellular fraction containing large particles (mitochondria, chloroplasts, lysosomes and microbodies), resembling the subcellular localization of ACBP1. To further investigate the subcellular localization of ACBP2, we fused ACBP2 translationally in-frame to GFP. Using particle gene bombardment, ACBP2-GFP and ACBP1-GFP fusion proteins were observed transiently expressed at the endoplasmic reticulum and at the plasma membrane in onion epidermal cells. GFP fusions with deletion derivatives of ACBP1 and ACBP2 lacking the transmembrane domain were impaired in membrane targeting. Our investigations also showed that when the transmembrane domain of ACBP1 or that of ACBP2 was fused with GFP, the fusion protein was targeted to the plasma membrane, thereby establishing their role in membrane targeting. The localization of ACBP1-GFP is consistent with our previous observations using immunoelectron microscopy whereby ACBP1 was localized to the plasma membrane and vesicles. We conclude that ACBP2, like ACBP1, is also a membrane protein that likely functions in membrane-associated acyl-CoA transfer / metabolism.

P-1101

Use of Marker Genes to Target Disease Resistance Gene Expression in Grape. ZHIJIAN LI, Subramanian Jayasankar, and D. J. Gray. Mid-Florida Research and Education Center, IFAS, University of Florida, 2725 Binion Road, Apopka, FL 32703. Email: zjli@ufl.edu

Pierce's disease (PD) results in devastating damage to the grape and wine industry and is caused by an insect-transmitted, xylem-limited bacterium, Xylella fastidiosa. In our effort to develop PD-resistant grapevines using transgenic technology, we employed an EGFP/NPTII fusion marker to monitor transgene expression. A series of transformation vectors containing various promoter constructs were developed. A large number of transgenic plants (cv. Thompson Seedless) were produced and GFP expression patterns in various plant tissues and organs monitored microscopically. Results indicated that up to 92% of independent transgenic plants containing a bi-directional dual promoter construct showed GFP expression that was predominantly localized in the vascular tissues. On the contrary, up to 78% of the transgenic plants containing a uni-directional promoter construct produced GFP expression that was mainly localized in the epidermal cells or non-vascular tissues. In addition, the levels of transgene expression were dramatically boosted by using bi-directional promoter design strategy. An antimicrobial lytic peptide gene Shiva-1 was incorporated into the novel promoter construct. Production of Shiva-1 protein in leaf and xylem sap samples was detected using a newly-developed ELISA method. The significance of this research in developing PD-resistance in grape will be discussed.

P-1102

Quantitative Measurements of Stable and Mosaic GFP Expression in Transformed Tobacco Leaves Using Flow Cytometry. ZLATA LUTHAR, Katarina Rudolf, Manja-Tina Bastar, Suzana Škof, and Borut Bohanec. Centre for Plant Biotechnology and Breeding, Biotechnical Faculty, Jamnikarjeva 101, 1111 Ljubljana, Slovenia. E-mail: zlata.luthar@bf.uni-lj.si

An improved protocol was developed to determine quantitative single cell fluorescence in GFP transformed tobacco leaf protoplasts as measured by multiparameter flow cytometry. Quantifications of fluorescence using fluorimetric methods are limited to average expression in tissues and cannot be assessed in single cells, while using flow cytometry quantitative data of single-cell origin are available. Fluorescent protoplasts and those expressing only background autofluorescence were separated using three parameter analysis. For clustered sub-populations, relative fluorescence intensity and proportions of cells with expressed or non-expressed fluorescence can be measured. Mosaic expression of GFP driven by the 35S promotor was measured in T2 and T3 tobacco plants with high numbers of integrated copies. Both stable expression or mosaicism were expressed in both generations, and large differences were found among lines and individual plants. These findings will be discussed in relation to gene expression and transgene silencing.

P-1103

Comparative Expression Analysis of Caulimovirus Full-length Transcript (FLt) and Subgenomic Transcript (Sgt) Promoters in Plants and Microbes. INDU B. MAITI, Sitakanta Pattanaik, Somnath Bhattacharyya, and Nrisingha Dey. Molecular Plant Virology and Plant Genetic Engineering Laboratory, Tobacco and Health Research institute, University of Kentucky, Lexington, KY 40546–0236. E-mail: imaiti@pop.uky.edu

We have analyzed genetic promoters for the full-length transcript (FLt) and subgenomic transcript from six different members of the Caulimoviridae family: Cauliflower mosaic virus (CaMV), Figwort mosaic virus (FMV), Peanut chlorotic streak virus (PClSV), Mirabilis mosaic virus (MMV), Cassava vein mosaic virus (CVMV) and Strawberry vein banding virus (SVBV). These caulimovirus-promoters have little sequence homology except some small regulatory motifs. Chimeric constructs of these promoters with GUS reporter gene were electroporated in tobacco (Xanthi) and maize (BMS) protoplasts to compare their strength. Expression pattern of these promoters was also assayed in stably transformed transgenic tobacco plants (Nicotiana tabacum, i/> cv. Samsun NN) and in E. coli cells. Strength of the MMV FLt promoter was highest in tobacco whereas the CaMV 35S promoter showed maximal activity in maize compared to other promoters analyzed. The MMV Sgt promoter fragment is a strong constitutive promoter, with strength comparable to that of the MMV FLt promoter. The MMV Sgt promoter and FMV Sgt promoter also demonstrated much greater activity compared to the CaMV 19S promoter and CaMV 35S promoter both in tobacco protoplasts and in transgenic tobacco plants. All caulimovirus-promoters tested gave a significant GUS activity in E. coli. The expression level of PClSV FLt promoter giving highest activity in E. coli was 400–450 fold more than that of CaMV 35S promoter. These promoters are very useful for expressing foreign genes in transgenic plants. There is very limited sequence homology among the caulimovirus-promoters although they are functionally analogous. For metabolic engineering, expression of multiple genes in a single cell will be necessary. The use of different promoters with non-homologous sequences may be useful in order to avoid genetic instability due to recombination between identical promoter sequences.

P-1104

Biotechnology for Answering Space Biology Questions. MICHAEL S. MANAK, Anna-Lisa Paul, Robert J. Ferl. Plant Molecular and Cellular Biology, University of Florida. Fifield Hall, Gainesville, FL 32611–0690. E-mail: manak@ufl.edu

Green fluorescent protein (GFP) is a reporter gene that provides the unique ability to collect in vivo data on gene expression patterns in a non-destructive manner. When coupled to a stress inducible promoter, such as the Arabidopsis Alcohol dehydrogenase (Adh) gene, GFP can be used to monitor a plants response to stress in real time. The in vivo stress response can be captured with digital video imaging equipment to detect the wavelength of light being admitted by GFP. Current data captured with this method include detailed digital footage of whole Arabidopsis plants showing stress induced gene expression patterns over a 20-day period. Arabidopsis transformed with an Adh promoter/GFP fusion construct show specific expression patterns unique to each environmental stress applied, hypoxia, cold, drought, and ABA. In addition to these known stresses, the Adh promoter/GFP fusion plants have been used to investigate the gene expression effects of low atmospheric pressures that may be experienced by plants in extraterrestrial habitats. Several other Arabidopsis promoters (ChiB, FAD7, HSP18.2, NR1, RD29A, and GSH2) were fused to GFP to monitor a network of responses (including ethylene, wounding, heat shock, nitrate metabolism, cold shock, and heavy metal uptake). The promoter/GFP fusions are biosensors that produce signals in Arabidopsis that can be captured with a digital video monitoring system and exported back to earth-based scientists via telemetry during extraterrestrial space exploration experiments.

P-1105

Production of Polyhydroxybutyrate, a Biodegradable Thermoplastic, in the Transformed Plastids of Tobacco. S. R. MIN, W. J. Jeong, and J. R. Liu. Plant Cell Biotechnology Lab., Korea Research Institute of Bioscience and Biotechnology, Taejon 305–333, Korea. E-mail: jrliu@mail.kribb.re.kr

For high-level expression of the polyhydroxybutyrate (PHB) operon from the soil-borne bacterium Alcaligenes eutrophus, E. coli containing the phage T7 RNA polymerase (T7 RNAP) gene in the chromosomal DNA was transformed with a vector containing the T7 promoter-PHB operon fusion and the ribosomal RNA 16S promoter (Prrn)-aadA gene fusion as a selectable marker against spectinomycin. The production of PHB inclusions in the transformed bacterial cells was confirmed by light microscope and gas chromatography. An analogous system for high-level production of PHB in the plastids of tobacco was established. The T7 RNAP gene fused to the tobacco chloroplast transit peptide sequence under the control of CaMV 35 promoter was introduced into tobacco by Agrobacterium-mediated transformation. An in vitro transcription assay confirmed that the transgenic tobacco produced a functional RNA polymerase. A plastid transformation vector containing the T7 promoter-PHB operon fusion and the Prrn-aadA gene fusion was constructed. Protoplasts isolated from leaf tissue of tobacco plant transformed with the T7 RNAP chimeric gene were incubated with DNA of the plastid transformation vector and cultured on shoot-inducing medium containing spectinomycin. Green colonies renenerated from protoplasts gave rise to green, spectinomycin-resistant, adventitious shoots, which were subsequently rooted. PCR analysis confirmed that the PHB operon was incorporated into the plastid genome. It is expected that transgenic tobacco plants containing nuclei with the T7 RNAP gene and plastids with the PHB operon are capable of producing PHB at unprecedentedly high levels.

P-1106

Abstract has been withdrawn

P-1107

Identification and Characterization of a Rice RNA- and Microtubule-binding Protein from Rice Endosperm Cells. D. G. MUENCH and S. D. X. Chuong. Department of Biological Sciences, University of Calgary, 2500 University Dr. NW, Calgary, AB, Canada, T2N 1N4. Email: dmuench@ucalgary.ca

The control of subcellular mRNA trafficking and localization is often mediated by protein factors that are directly or indirectly associated with the cytoskeleton. We report the identification and characterization of a rice seed protein that possesses both RNA and microtubule binding activities. In vitro UV-crosslinking assays indicated that this protein binds to all mRNA sequences tested, although there was evidence for preferential binding to RNAs that contained A-C nucleotide sequence motifs. The protein was purified to homogeneity using a two-step procedure, and amino acid sequencing identified it as the multifunctional protein (MFP), a peroxisomal enzyme known to possess a number of activities involved in the b-oxidation of fatty acids. The recombinant version of this rice MFP binds to RNA in UV-crosslinking and gel mobility shift experiments, co-sediments specifically with microtubules, and possesses at least two enzymatic activities involved in peroxisomal fatty acid b-oxidation. Taken together these data suggest that MFP has an important role in mRNA physiology in the cytoplasm, perhaps in regulating the localization or translation of mRNAs through an interaction with microtubules, in addition to its peroxisomal function.

P-1108

Potential Application of Hairy Roots as a Tool for Activation Tagging. T. MURANAKA, C. Nakajima, A. Kohara, and S. Yoshida. Plant Science Center, RIKEN, Wako, Saitama 351–0198 JAPAN. E-mail: muranaka@postman.riken.go.jp

Activation tagging, *Agrobacterium tumefaciens*-mediated transformation with a T-DNA that carries enhancer and/or promoter sequences, is used as a gene discovery tool based on gain of function. However, the application of this method is restricted to a model plant such as *Arabidopsis* because it needs large number of transgenic plants for selection. Hairy roots are coursed to emerge at the inoculated site of various dicotyledonous plants in a high efficiency by infection with *A. rhizogenes*. In other words, *A. rhizogenes*-mediated transformation is thought to be a high-throughput system to obtain large number of transgenic root clones. We have already demonstrated that the promoter of the *parAt* gene of tobacco is expressed throughout the tissues of hairy roots of various plants including tobacco and *Arabidopsis*. In this presentation, we show the potential application of hairy roots as a tool for activation tagging by using *A. rhizogenes* harboring GFP as a non-disrupted selection marker and *parAt* gene promoter.

P-1109

Use of Matrix Attachment Regions (MARs) to Enhance Transgene Expression in Maize Cells and Callus Transformed by Biolistic Method. THANH-TUYEN NGUYEN, George C. Allen, Tai Minh, Connie M. Rowinski, and William F. Thompson. Department of Botany, North Carolina State University, Raleigh, NC 27695–7612. E-mail: tuyen_nguyen@ncsu.edu

We have previously shown that Matrix Attachment Regions (MARs) sequences flanking a transgene increase and stabilize transgene expression in transgenic tobacco plants and cell lines, most likely by reducing gene silencing. We aim to study the roles of MARs in reducing gene silencing and thus, possibly increasing the predictability and stability of transgene expression in Black Mexican Sweet (BMS) maize cells and Hi II callus. BMS cells and Hi II callus were co-bombarded with a reporter plasmid carrying GUS gene sequence driven by the maize ubiquitin promoter/intron (Ubi P/I-GUS-nosT) and a hygromycin selectable marker construct driven by the mannopine synthase system (mas-hptII-nosT). Transgene expression in the transformants is being compared for reporter constructs having MARs (pTN5) and without MARs (pAGM606) flanking the transgene. We will report data on transgene copy numbers with and without the MARs flanking sequences, effects of varying the ratio of the reporter to selectable marker plasmids, and the interaction of MARs with promoters of different strength (i.e. maize ubiquitin vs. 35S promoter)

P-1110

In Vivo Monitoring of Genetic Transformation in Tumors Induced by *Agrobacterium tumefaciens* in Plants. M. Dabauza, M. M. López, L. PEÑA. Department Plant Protection and Biotechnology, Instituto Valenciano de Investigaciones Agrarias, Apartado Oficial, 46113-Moncada, Valencia, Spain. E-mail: lpenya@ivia.es

Agrobacterium tumefaciens is a soil phytopathogenic bacterium that elicits the crown gall disease in most dicotiledoneous plants. The disease is characterized by the formation of tumors which typically occur on the plant stem. The basic mechanism of tumorigenesis involves the transfer of a piece of its tumor-inducing (Ti) plasmid, the transferred DNA (T-DNA), into the plant cell. Expression of oncogenes from the T-DNA causes overproduction of auxins and cytokinins and subsequently crown galls develop at infection sites where unregulated plant cell division occurs. In contrast to our extensive knowledge of the molecular events and function of bacterial and plant genes involved in the transformation process, it is not known whether tumor growth and development in a given host results from transformation of most tumor cells in infected tissues or most of them are non-transformed cells habituated to increased phytohormone levels produced by a few transformed cells. Southern blot analyses of integrated T-DNA in uncloned and cloned axenic tumor tissues as well as using of a binary plasmid harboring a *uidA* transgene in an *Agrobacterium* strain carrying also its wild type Ti plasmid have revealed highly divergent data about the proportion of transformed cells in tumors, from less than 1% in some studies to almost 100% in a recent report. We have used the *A. tumefaciens* wild type strain A281 with a binary plasmid containing a green fluorescent protein (*gfp*) transgene and analyze GFP positive and negative tissues by Southern blot to investigate early events in transformation and tumor formation and to localize *in vivo* transgenic sectors in tumors induced in the stem of tomato and sweet pepper plants. Results of these experiments will be shown in detail.

P-1111

Nucleolar Dominance: Uniparental Gene Silencing on a Multi-megabase Scale in Genetic Hybrids. CRAIG S. PIKAARD, Michelle Lewis, and Richard Lawrence. Biology Department, Washington University, Saint Louis, MO 63130. E-mail: pikaard@biology.wustl.edu

The failure of one parent's chromosomes to organize one or more nucleoli in an interspecific hybrid is an epigenetic phenomenon known as nucleolar dominance. Changes in DNA methylation and histone modification play a role in silencing the inactive set of rRNA genes, which is the basis for the phenomenon and which takes place on a scale of millions of basepairs. Aided by genome sequence data for Arabidopsis thaliana, we have mapped the extent of nucleolar dominance-induced silencing in Arabidopsis suecica, the allotetraploid hybrid of A. thaliana and A. arenosa. Our data show that chromosomal gene silencing is restricted to the rRNA genes at the nucleolus organizer regions (NORs) and does not spread to adjacent genes as near as 3kb. We have succeeded in genetically transforming A. suecica and have created transgenic hybrid plants expressing constructs to induce the silening (by RNA interference) of all known DNA methyltransferases, histone deacetylases and methylcytosine binding proteins. The effects of knocking down these, and other, chromatin modifying activities on nucleolar dominance will be presented.

P-1112

Ectopic Expression of Oleosin in *Arabidopsis thaliana* Shows the Protein Trafficking Along the Endoplasmic Recticulum. A. J. REID, I. R. Moore, and M. M. Moloney. Dept. of Biological Sciences, University of Calgary, Calgary, AB, T2N 1N4 and Dept. of Plant Sciences, University of Oxford, Oxford, OX1 3RB. E-mail: ajreid@ucalgary.ca

Fusions of green fluorescent protein (GFP) and *Brassica napus* oleosin were ectopically expressed in transgenic *Arabidopsis thaliana* plants. A transient expression system was designed to observe real-time movement of the oleosin-GFP bodies moving along the endoplasmic reticulum (ER) in plant epidermal cells. This is the first time that oleosin has been shown to be a dynamic protein, trafficking along the ER at rates comparable to other organelles such as Golgi bodies and mitochondria. Oleosin-GFP bodies were observed to range in size and speed from fast-moving small bodies (0.5 to 2 mm) to slow-moving large clusters (5 to 10 mm). These larger bodies appear to be stabilized within the cortical ER membrane, with the smaller bodies moving rapidly along the ER, and at times passing through the large clusters. When compared to the seed-specific expression of oleosin-GFP, the constitutively expressing oleosin-GFP embryos displayed identical localization of oleosin. The size and shape of oil bodies were also identical in the heart, torpedo and mature stages. However, prior to triacylglycerol (TAG) accumulation, the constitutive oleosin-GFP embryos possessed irregularly shaped oleosin-GFP bodies, similar to those seen in non-oil tissues. This provides an interesting clue to the timing of oleosin accumulation on oil bodies.

P-1113

Expression of an Isopentenyl Transferase Gene, Under the Control of a Senescence-specific Promoter, in Transgenic Rice Plants. LEILA RUBIA*, J. de Palma**, B. Ghareyazie***, G. S. Khush**, N. W. Scott*, M. Kaminek****, P. Dobrev****, J. Bennett** and M. C. Elliott*. *The Norman Borlaug Institute, De Montfort University, Scraptoft, Leicester, LE7 9SU, UK; **International Rice Research Institute, MCPO Box 3127, 1271 Makati City, Los Banos, Laguna, Manila, Philippines; ***Agricultural Biotechnology Research Institute of Iran, Karaj, Iran; and ****Institute of Experimental Botany, Academy of Sciences of the Czech Republic, Rozvojova 135, 16502, Praha 6, Czech Republic. E-mail: lrubia@dmu.ac.uk

The *Agrobacterium* T_1 plasmid isopentenyl transferase gene (*ipt*) encodes an enzyme which catalyses the biosynthesis of isopentenyl-type cytokinins during crown gall formation. Early attempts to manipulate plant growth involved the expression of the gene under the control of constitutive promoters. Usually abnormal plants with delayed leaf senescence were formed. Although the abnormalities were not welcome in crop plants, the delayed senescence could lead to an increase in assimilate production. To investigate the feasibility of this approach to crop improvement, the *ipt* gene, under the control of a senescence-specific promoter, *SAG12* from *Arabidopsis thaliana*, has been introduced into New Plant Type rice. PCR and Southern blot analyses were used to confirm the integration of the *ipt* gene into the genome of eight independent transgenic lines and their 221 clones. In four of these lines the expression of the *ipt* gene was detected in the senescing leaves but not in pre-senescing or senesced leaves. However, the *ipt* gene was expressed in the non-senescing leaves of three of the other lines, indicating that the *Arabidopsis SAG12* promoter sometimes failed to deliver the desired expression pattern in the rice background.

P-1114

Evaluating Transgene Expression with Quantitative Real-time PCR. M. A. SCHMIDT and W. A. Parrott. Crop and Soil Science Dept. University of Georgia, Athens, GA 30605. E-mail:schmidt@uga.edu

The 5'nuclease quantitative real-time TaqMan™ PCR methodology was evaluated for its effectiveness in measuring transgene expression levels in plant tissue. In this study the level of transgene expression for the genes that confer resistance to the antibiotics hygromycin and kanamycin were quantitated in soybean [*Glycine max* (L.) Merrill] and white clover [*Trifollium repens* (L.)], respectively, by a multiplexed fluorogenic real-time PCR assay. Transgene expression was estimated as gene expression levels normalized by the expression of ribosomal transcripts used as an internal standard control. The simultaneous amplification of cDNAs for both the target gene and the endogenous control gene negated the time-consuming and laborious need to produce an external standard curve to quantify samples against, eliminated the need to quantitate the amount of starting transcripts added to the PCR reaction and minimized sample-to-sample variation. A correlation between transgene expression levels determined by multiplexed real-time PCR system and conventional northern blot hybridization analysis was made. TaqMan™ technology has the distinct adavantage of using minimal starting material compared to conventional expression assays. Hence, through the use of a TaqMan™-based expression assay, transformants that are expressing the transgene(s) in an optimal way can rapidly be identified and subsequent efforts in plant regeneration could focus only on expressing lines. This assay offers a higher level of specificity than standard quantitative PCR-based techniques since it is based on a fluorogenic probe and two primers designed against the target gene.

P-1115

Isolation and Characterisation of a Proline Rich Hydrophobic Hybrid Protein from Sugar Beet. NIGEL W. SCOTT, M. R. Fowler, A. Slater, A. R. Milan, W. M. Norton, and M. C. Elliott. The Norman Borlaug Institute for Plant Science Research, De Montfort University, Scraptoft, Leicester, LE7 9SU, U.K. E-mail: nws@dmu.ac.uk

The Norman Borlaug Institute has developed a strategy for enhancement of sugar beet sucrose yields while minimising soil tare. Gene manipulation techniques will be used to alter the anatomy of the *Beta vulgaris* storage organ in order to optimise the structure for sucrose storage. To facilitate this approach sequences have been isolated that will be useful as molecular probes or as the source of promoters to target gene expression to specific cells within the storage organ. A member of the proline rich hydrophobic hybrid protein family (*RS1*) has been isolated from sugar beet and characterised. The expression of this gene in the sugar beet storage organ during development has been studied using Northern blot hybridisation and whole mount *in situ* hybridisation (WISH). The protein is expressed in a region of high sucrose concentration, in parenchymatous cells adjacent to the supra numerary cambia. A full genomic clone has been isolated for this sequence and a deletion analysis of the promoter has been carried out. The promoter region fragments have been fused to *gusA* for expression analyses. The expression patterns of these fragments in transgenic tobacco and carrot have been compared with those of other promoter: : *gusA* fusions. The results suggest that the product of *RSI* is, indeed, a cell wall component and that its expression is enhanced under conditions of mechanical stress.

P-1116

Molecular Biology of Microspore Wall Development in Wheat (*Triticum aestivum* L.). GOPALAN SELVARAJ, Aiming Wang, Qun Xia, Wenshuang Xie, Tim Dumonceaux, Jitao Zou, and Raju Datla. Plant Biotechnology Institute, National Research Council of Canada, Saskatoon, Canada S7N 0W9. E-mail: GOPALAN.SELVARAJ@NRC.CA

Common bread wheat (*Triticum aestivum* L.; hexaploid AABBDD genome) is a genetically complex plant. In spite of its importance, several aspects of wheat biology remain poorly understood and the molecular biology of male gametophyte development is one of them. We have isolated three apparently homeologous genes whose expression is specific to developing anthers. Transcripts of these could not be found in northern blots of RNA from root, stem, leaf, ovary or glume samples. These eight-exon genes, found at one copy per haploid complement, are predicted to encode a ~ 58 kDa polypeptide. *In situ* RNA and protein localization studies showed that the protein was produced in tapetum cells and that it was associated with thickening of the exine during microspore wall development after resolution of the tetrads into free microspores. The function of these genes in microspore development, as inferred from cellular, molecular and biochemical studies, will be presented.

P-1117

Array-based Gene Expression Analysis of Citrus Leaf Response to Weevil Feeding and Mechanical Damage. R.G. SHATTERS, JR., P. Dang, X. Sinisterra, M. G. Bausher. USDA, ARS, U. S. Horticultural Research Laboratory, Fort Pierce, FL 34945. E-mail:rshatters@ushrl.ars.usda.gov

Array-based gene expression allows rapid screening of a large number of plant genes for differential expression in response to environmental stimuli. We have used an array of 384 cDNAs randomly selected from a whole seedling EST project on sweet orange (*Citrus sinensis* L. Osbeck) for differential gene expression analysis in leaf tissue. Comparisons were performed with young expanding leaves undamaged, and damaged by either Diaprepes root weevil adult feeding or mechanical simulation of feeding and mature source leaves undamaged and damaged by mechanical feeding simulation. In young leaves, the majority of the damage-induced changes in gene expression were similar for both types of damage. Damage induced increases in transcript abundance was observed for: a group of metallothionein-related sequences, a GDSL-lipase/hydrolase, a carbonate dehydratase, and a harpin-induced related sequence. Genes showing significant reductions in associated transcript levels in response to the damages included sequences similar to: myo-inositol-1-phosphate synthase, UDP-glucose 4-epimerase, a heat shock 18.1 kDa protein, a succinate dehydrogenase subunit, a 14–3–3 protein, and caffeic acid O-methyl transferase. There were four sequences that were induced primarily in response to insect feeding that were similar to: pectin acetylesterase, cysteine proteinase, endoxyloglucan transferase, and catalase. Very few changes in mature leaves in response to the mechanical damage were observed, and the changes that occurred in the young leaves often resulted in expression levels of the gene that were similar to that in mature undamaged leaves. Although the array contained seven sequences encoding putative pr-proteins, and at least one related to known pathogen induced sequence, the transcript level of these did not respond to any of the damage treatments. Differential expression results will be presented with respect to the effected metabolic pathways, and show the presence of previously unknown relationships" between gene expression and stress response.

P-1117A

Expression of Two Omega-3 Fatty Acid Desaturase Transgenes (NtFAD3 and AtFAD7) in Rice Plants Cotransformed by Particle Bombardment. T. SHIMADA, Y. Wakita, M. Otani, H. Tanno, and K. Iba. Research Institute of Agricultural Resources, Ishikawa Agricultural College, Suematsu, Nonoichi, Ishikawa 921–8836, JAPAN. E-mail: shimada@ishikawa-c.ac.jp

Three genes carried on three separate plasmids were co-transformed in japonica rice (*Oryza sativa* cv. Notohikari) by particle bombardment. A microsomal omega-3 fatty acid desaturase gene from tobacco (*NtFAD3*) and a chloroplast omega-3 fatty acid desaturase gene isolated from *Arabidopsis thaliana* (*AtFAD7*) were controlled by CaMV35S promoter and a *bar* gene as a selectable marker gene was controlled by rice actin 1 promoter. We obtained some transgenic lines with only *bar* gene, *bar* and *NtFAD3* or *AtFAD7* genes and *bar*, *NtFAD3* and *AtFAD7* genes, evaluated by Southern analysis. Two of seven transgenic lines with both *NtFAD3* and *AtFAD7* showed the modified fatty acid composition in seedling roots and leaves of next generation (R1): Linoleic acid (18:2) contents decreased and linolenic acid (18:3) contents increased significantly. However, the remains of five lines showed the same composition as non-transgenic plants, indicating the *NtFAD3* and *AtFAD7* did not express in the transgenic plants. In the next generation (R2), one (#F37–32) of two lines contained decreasing linoleic acid and increasing linolenic acid, while the other (#F37–15) showed no difference with non-transgenic plants. The Northern analysis reveled that #F37–32 transcribed *NtFAD3* and *AtFAD7* in both R1 and R2 generations. However, #F37–15 did not show mRNA of *AtFAD7* in R1 generation and of *NtFAD3* and *AtFAD7* in both R1 and R2 generations, although only *NtFAD3* expressed in R1 generation. This results could reflect the epigenetic phenomena such as homology-dependent transcriptional silencing caused by the multiple copy number, integrative fragmentation and rearrangements of the transgenes.

P-1118

Regulation of Expression of OsIAA1, an Aux/IAA Gene from Rice and Its Localization. J. K. THAKUR, A. K. Tyagi, and J. P. Khurana. Department of Plant Molecular Biology, Delhi University South Campus, New Dehli-10021, INDIA. Email: jitu707@rediffmail.com

The *Aux/IAA* class of genes are rapidly induced by exogenous auxins and have been characterized extensively from many dicot species like *Arabidopsis*, *Glycine max* and *Pisum sativum*. We have isolated and characterized rice (*Oryza sativa* L. subsp. *Indica*) *OsIAA1* cDNA and gene as a monocot member of the *Aux/IAA* gene family. The structural part of the gene is 2.4 kb long with 5 introns and 6 exons which corresponds to 1064 bases long cDNA. The predicted amino acid sequence of OsIAA1 corresponds to a protein of *ca* 26 kDa, which harbors all the four characteristic domains known to be conserved in Aux/IAA proteins. The conservation of these *Aux/IAA* genes indicates essentially a similar mode of action of auxin in monocots and dicots. There are two NLSs, one bipartite and the other resembling SV40 NLS. In this study we fused GUS coding sequence with deleted or complete regions of *OsIAA1* cDNA. Both the NLSs are able to target the protein to the nucleus, the bipartite being more efficient and effective. At present it is not ascertained whether the Ser residue present near NLSs are important for nuclear localization of the protein. Northern analysis revealed that the *OsIAA1* transcript levels decrease in the excised coleoptile segments on auxin starvation, and the level is restored when auxin is supplemented; the increase in *OsIAA1* transcript level was apparent within 15 to 30 min of auxin application. The auxin-induced *OsIAA1* expression appears to be correlated with the elongation of excised coleoptile segments. In light-grown rice seedlings, *OsIAA1* is preferentially expressed in roots and basal segment of the seedling, whereas in the etiolated rice seedlings, the *OsIAA1* transcripts are most abundant in the coleoptile. A comparative analysis in light- and dark-grown seedling tissues indicates that the *OsIAA1* transcript levels decrease on illumination. Even illumination with blue or red light also decreases the transcript level. This indicates blue or red light receptors (say cry or phy) do have some correlation with OsIAA1. We have raised antimice antisera against purified His_6-OsIAA1 protein. Efforts are in progress to find other proteins that interact with OsIAA1 and we are also trying to silence *OsIAA1* by RNA interference which will tell its function.

P-1119

Evaluation and Analysis of NeIF-4A10 Promoter Expression in Plants. LINING TIAN[1], Daniel C. W. Brown[1], Keqiang Wu[2*], Marysia Latoszek-Green[1], Susan Sibbald[1], Ming Hu[2], Brian Miki[2]. [1]Southern Crop Protection and Food Research Centre, Agriculture and Agri-Food Canada, 1391 Sandford St, London, Ontario, Canada N5V 4T3 and [2]Eastern Cereal and Oilseed Research Centre, Agriculture and Agri-Food Canada, Ottawa, Ontario, Canada K1A 0C6 (*Current address: Biology Department, West Virginia University, PO Box 6057, Morgantown, WV 26506–6057). Email: browndc@em.agr.ca

The NeIF-4A10 gene codes for an RNA helicase that is important for the binding of the 40S ribosomal subunit to the 5'end of mRNA for translation initiation. Presumably, this is a housekeeping gene function needed in all cell types. A preliminary analysis of this promoter sequence has been done (Mandel et al., 1995, Plant Mol. Biol. 29: 995–1004). In extending the work, studies were carried out to evaluate its expression in different plant species and also to further functionally analyse NeIF-4A10 promoter, especially the proximal regions. The NeIF-4A10 promoter was found to express in a wide range of dicotyledon plants and gymnosperm plants, but express at low levels in several monocotyledon plants. Removal of the intron which is about 1 kb from the leader sequence did not show a reduced effect on the gene expression in tobacco leaves and alfalfa cell suspension culture following bombardment, indicating that the intron sequence is not important for the promoter expression. Deletion analysis indicated that the −188 bp to −73 bp region, relative to the transcriptional start site, may contain transcriptional enhancer activity. Linker-scan mutagenesis within this region also indicated existence of transcriptional enhancer activity. Removal of the leader sequence from the promoter reduced promoter activity, suggesting the importance of the leader for promoter expression. The promoter with a deletion to the −73 bp site still showed constitutive expression in transgenic tobacco plants, indicating the elements for constitutive expression reside within this region.

P-1120

Modeling Loblolly Pine Arginase Regulation Using an *In Vitro* Approach. CHRISTOPHER D. TODD and David J. Gifford. Department of Biological Sciences, University of Alberta, Edmonton, Alberta, Canada, T6G–2E9.

In mature seeds of loblolly pine (*Pinus taeda* L.), greater than 85% of the storage protein reserve is located in the megagametophyte; the remainder is distributed in the embryo. By far the largest amount of nitrogen in these proteins is stored as arginine, a very efficient nitrogen storage compound. Following seed germination, storage protein breakdown in the megagametophyte and in the seedling results in a large increase in the seedling's free arginine pool. This arginine is hydrolyzed in the seedling by the enzyme arginase (E.C. 3.5.3.1), which is under strong developmental control. Using an *in vitro* culture system to address the separate impacts of the seedling and megagametophyte tissues on arginase enzyme activity, protein levels and patterns of gene expression we have shown that arginase regulation in the cotyledons is initiated by the seedling itself and not by the megagametophyte as previously proposed. Removal of the megagametophyte tissue from seedlings cultured in its presenced caused an immediate decrease in arginase transcript levels. Addition of arginine to embryos cultured in the absence of the megagametophyte caused upregulation of arginase gene expression. Results obtained using this *in vitro* approach have allowed us to propose a model of arginase regulation whereby seedling derived arginine initiates arginase expression and free arginine from the megagametophyte acts in a positive manner to further increase arginase transcript abundance.

P-1121

Improvement of Barley Resistance to BYDV by *Agrobacterium tumefaciens*-mediated Transformation with Intron-spliced 'Hairpin RNA' (ihpRNA) and scFv-antibodies. V. V. VALKOV[1], V. Formitcheva[2], C. Marthe[1], C. Münnich[1], J. Schubert[2], J. Kumlehn[1], F. Altpeter[3], and U. Conrad[1]. [1]Institute for Plant Genetics and Crop Research, Corrensstrasse 3, 06466 Gatersleben, Germany; [2]Institute for Resistance Research and Pathogen Diagnostics, Theodor-Roemer Weg 4, 06449 Aschersleben, Germany; and [3]University of Florida, Institute for Food and Agricultural Sciences, Agronomy Unit, 2191 McCarty Hall, PO Box 110300, Gainesville, FL 32611–0300. E-mail: VALKOV@IPK-GATERSLEBEN.DE

Barley Yellow Dwarf Virus (BYDV) is the most serious and widespread virus of cereals world-wide. In barley it causes up to 40% yield losses and significant decrease of grain quality. Sources of natural resistance are rare, and the application of insecticides to prevent virus spreading by aphids is economically and environmentally undesirable. A BYDV-ASL isolate was sequenced and a small part of the 3' end was amplified by PCR. The resulting fragment was cloned into a binary vector in sense, antisenese and both sens-antisense orientation. Other vectors were designed as sense-intron-antisense in order to produce self-complementary RNA (hairpin structure) which might induce gene silencing. The transgenes were under the control of either the constitutive Actin promoter or a phloem-specific promoter. The constructs were transferred to the Agrobacterium tumefaciens strain AGL1. Immature barley embryos were transformed and selection was conducted on Hygromycin. Another way of improving virus resistance to BYDV based upon expression of virus RNA-polymerase specific scFv antibodies was attempted. The DNA sequence for the scFv antibody was engineered according to data obtained from the BYDV analysis. Barley plants were transformed with a construct carrying the sequence for scFv antibody to determine whether the expressed antibody is effective in blocking the virus RNA-polymerase. The aim of the presented work is to investigate mechanisms of gene silencing and virus resistance, as well as to provide reliable virus immunity that is stably inherited.

P-1122

Arabidopsis Genome as a Source of Promoters Active in Monocot Crops. CHUAN-YIN WU, Emilio Margolles-Clark, Anthony Trieu, Ed Kiegle, Kristofer Munson, and Richard Flavell. Ceres, Inc., 3007 Malibu Canyon Road, Malibu, CA 90265. E-mail: cyinwu@ceres-inc.com

There are relatively few evaluated promoters available for use in research and genetic engineering of monocot crops. Promoters with different activity or tissue/cell type specificity are expected to be in increasing demand for functional analysis of genes and crop improvement. Since the whole genome sequence of Arabidopsis is available and data from microarrays for various genes are increasingly documented, one can easily find the 5' upstream region of a given gene with a known expression profile and clone it using the PCR. We were interested in testing if the Arabidopsis genome can be a source to efficiently identify promoters active in monocot crops. We chose twenty-three Arabidopsis genes based on their expression profile and cloned their 5' upstream regions. The cloned sequences were fused to the green or yellow fluorescent protein coding sequence and tested first in rice suspension culture-derived protoplasts using electroporation. Eight promoters were found active in protoplasts as compared with controls. We will demonstrate that easy cloning of Arabidopsis genomic sequences, combined with testing in rice can be a useful approach to discover promoters for use in monocot crops.

P-1123

Evaluation of the Arabidopsis GAI/gai gene(s) in Transgenic Maize. DEPING XU, Xiaolan Duan, Haiyin Wang, Xiaomu Niu, Dwight Tomes, Sarah Collinson, and Greg Edmeades. Research Center, Pioneer Hi-Bred International, Inc., A DuPont Company, Johnston, IA 50131. E-mail: Deping.Xu@Pioneer.com

Dwarfing mutant genes have the great potential to be used as molecular tools for modification of crop stature. Currently, we are evaluating several dwarfing mutant genes from Arabidopsis for their potential utility in modifying maize plant height. The wild type GAI and mutant gai (*gibberellic acid insensitive*) genes from *Arabidopsis* have been used for maize transformation. Transgenic maize plants containing the GAI or gai gene have been evaluated for several generations and events with consistent height reduction have been identified for further characterization. In summary, transgenic maize events with the GAI gene driven by the rice actin1 promoter showed the most significant and consistent height reduction (5%-15%). Transgenic plants also have reduced leaf length (10%-20%) and increased stalk diameter (5%-10%). However, ear height was increased in many events. No significant effect of the GAI gene was detected for the following traits: ear leaf chlorophyll, days to 50% pollen shed, anthesis-silking interval (ASI), and total leaf number. Evaluation of other dwarfing genes in both transgenic Arabidopsis and maize plants are in progress. We will present our detailed results in the poster.

P-1124

CRW Control Using Pentin-1 Gene: Comparison of Bombardment- and *Agrobacterium*-mediated Transformation. J. ZHANG, V. Gustafson, D. Xu, D. Liu, B. Li, K. Nour, J. Swenson, K. Hagemann, L. Higgins, M. Peters, A. Pascual, S. Jayne, and D. Moellenbeck. Pioneer Hi-Bred International Inc., Johnston, IA 50131. E-mail: jian.zhang@pioneer.com

Corn rootworm (CRW) is the major insect pest of dent corn causing an estimated $1 billion in crop loss each year in the US. Pentin-1 is a CRW active protein isolated from the seeds of *Pentaclethra macroloba*, a tropical legume. The particle gun method was the first approach used for production of fertile transgenic corn plants in the late 1980's. This technology is applied at Pioneer using freshly isolated immature embryos for T0 event production today. Because monocot plants are not natural hosts for *Agrobacterium tumefaciens*, the development of an *Agrobacterium*-mediated transformation protocol has lagged behind those for dicotyledons. In 1996, transgenic plants from freshly isolated immature embryos of corn infected by *Agrobacterium* were obtained by Japan Tobacco. This technology has been adopted into Pioneer corn transformation system. The Insect Resistance Transformation group uses both the particle gun and *Agrobacterium*-mediated transformation methods to produce corn transgenic events for Pentin-1 gene evaluation. Here we compared the two genetic transformation methods in terms of transformation frequency, transgene expression and gene efficacy in bioassay.

P-1125

In Vitro Conservation of *Cedrela fissilis* a Tree Native to the Brazilian Atlantic Forest. Eduardo da Costa Nunes*, ERICA E. BENSON**, Ana Maria Viana*. *Plant Conservation Group, School of Science and Engineering, University of Abertay Dundee, Bell Street, Dundee, DD1 1HG, Scotland, UK; **Departamento de Botânica, Universidade Federal de Santa Catarina, 88040–900 Florianópolis, SC, Brasil. E-mail: amarna@floripa.com.br

Few studies have been undertaken regarding the *in vitro* conservation of Brazilian tropical forest trees; this is especially the case for the Meliaceae. Two *in vitro* conservation methods are presented for the *ex situ* conservation of germplasm from *Cedrela fissilis*, an economically important tree of the Brazilian Atlantic Forest. The first is medium-term storage, at 25° C, of artificial seeds comprising alginate-encapsulated vegetative propagules. Maximum post-storage (3 months) recovery of 96–100% was achieved for encapsulated shoot tips and cotyledonary nodal segments stored on water-solidified agar (at 0.4 to 0.7% w/v). Encapsulated shoot tips stored for 6 months on 0.4% (w/v) agar showed the highest survival rates (44%). In the second, seeds of *C. fissilis* were successful cryopreserved (with 100% survival) after direct immersion in liquid nitrogen. *Ex situ* storage procedures are now available for the medium, to long-term conservation of *C. fissilis*. These approaches offer new opportunities for the conservation, sustainable management and utilization of this valuable fast growing Brazilian timber tree.

P-1126

Somatic Embryogenesis in Common Ash. M. CAPUANA and A. Di Marco. Institute of Forest Tree Breeding, 50134 Firenze, Italy. E-mail: capuana@imgpf.fi.cnr.it

These are the first achievements on somatic embryogenesis in common ash (*Fraxinus excelsior* L.), a very important species for several European countries, where it is widely planted for wood production and landscaping. Experiments on somatic embryogenesis induction were carried out on zygotic embryos at different phases of development and maturation. For this purpose, seeds were collected after complete formation of cotyledons as well as in the following weeks during their maturation on the tree. A second phase of experiments was realised with embryos collected from seeds that, after have been collected from trees in autumn, were submitted to a warming/chilling treatment for dormancy breaking, and then sampled at regular intervals. The embryos were extracted from sterilised seeds, and the axes were cultured on modified (half-strength) MS (Murashige and Skoog) medium enriched with combinations of 2,4-D, BA and thidiazuron. Embryogenic tissues were obtained from embryos collected at an incomplete maturation phase and cultured on 2,4-D and BA-containing medium. Somatic embryos developed from embryogenic tissue and matured up to the cotyledonary stage, but they did fail to germinate. Embryos extracted from seeds during the warming/chilling treatment gave lower response and somatic embryogenesis was obtained only sporadically. Organogenetic phenomena were observed on several explants as well, as a response to the hormonal treatments.

P-1127

Genetic Transformation of White Poplar (*Populus alba* L.) with a Vitreoscilla Hemoglobin (VHb)-encoding Gene and Evaluation of Growth Rate and Biomass Production. M. CONFALONIERI, S. Reggi, L. Anfosso, A. Balestrazzi, P. Calligari, C. Bongiorni, and C. Fogher. Istituto Sperimentale Colture Foraggere, Lodi, I-26900, Italy. Istituto Sperimentale Pioppicoltura, Casale Monferrato; Plantechno Srl, Casalmaggiore; Dipartimento Genetica e Microbiologia, Pavia; Istituto Botanica e Genetica Vegetale, Piacenza, Italy. E-mail: iscfchem@telware.it

The aim of this study was to obtain transgenic white poplar with enhanced growth and biomass production. Internodal stem segments of *Populus alba* L. (cv. 'Villafranca') were co-cultivated with EHA105 disarmed *Agrobacterium tumefaciens* strain. The binary vector pBI-VHb contained the *vhb* gene for *Vitreoscilla* hemoglobin-like protein (VHb) and the neomycin phosphotransferase II (*nptII*) gene. Putative transgenic plantlets were regenerated from different calluses and selected on a medium containing kanamycin. Transformation was demonstrated by Southern and northern blot hybridization analyses. *In vitro* growth measurements showed that VHb expression in transgenic poplar plants did not significantly affect their growth patterns, including stem elongation, leaf enlargement, and stem and root dry weight biomass. However, two of the selected transgenic lines grown in greenhouse showed significantly higher values for plant height, stem diameter, number of nodes and internodes, and shoot biomass than the controls. Further studies are currently under way to characterize in more detail growth rate and biomass production of the selected transgenic poplars.

P-1128

Studies of Proteins in the Hybrid Aspen *Populus tremula x tremuloides* Believed to be Involved in Cellulose Synthesis. SORAYA DJERBI[1], Vincent Bulone[2], Anna Ohlsson[1], Torkel Berglund[1], Tuula T. Teeri[1], Kristina Blomqvist[1]. [1]Royal Institute of Technology, Department of Biotechnology, SCFAB, S-106 91 Stockholm, Sweden and [2]Centre de Recherche sur les Macromolécules Végétales, CNRS-UPR 5301, BP53, 38041 Grenoble cedex 9, France. E-mail: SORAYA@BIOCHEM.KTH.SE

Genetic fiber modification is an attractive possibility for e.g. the pulp and paper industry, where improved raw material consisting of longer and stronger fibers is desired. In a quest to find new tools of fiber modification our aim is to identify the components involved in cellulose synthesis from the hybrid aspen *Populus tremula x tremuloides*. Based on sequence similarity, five putative CesA genes were identified in an EST library from the wood forming tissue in aspen (**1**). The full-length coding sequences of the five CesA's were cloned and sequencing is in progress. Microarray analysis of the gene expression during different stages of xylogenesis revealed that at least two of the CesA isoenzymes are specifically involved in cellulose biosynthesis of the secondary cell wall formation (**2**). Furthermore, preliminary data shows that one of the genes is specifically induced during tension wood formation. Antibodies have been successfully raised against the catalytic domain of CesA and used as analytical tools in Western blot analysis, immunolocalisation-and immunoprecipitation studies. **1**. Sterky et al. (1998) Proc Natl Acad Sci USA, 95:13330 **2**. Hertzberg, M, Aspeborg, H, Schrader J, Andersson A, Erlandsson R, Blomqvist K, Bhalerao R, Uhlen M, Teeri TT, Lundeberg J, Sundberg B, Nilsson P, Sandberg G. A transcriptional roadmap to wood formation. Proc Natl Acad Sci USA. 2001 Dec 4;98(25):14732–7.

P-1129

Towards the Cloning of Early Flowering Genes in Tropical Tree Species. M. C. DORNELAS, A. P. M. Rodriguez and W. A. N. do Amaral. University of São Paulo, ESALQ, Dept. of Forest Sciences, Piracicaba, SP, Brazil. E-mail: mcdornel@carpa.ciagri.usp.br

Breeding programs for tropical tree species have limitations due to a long juvenile phase, therefore early flowering phenotypes are highly desired. The transition to reproductive development has been intensively studied at the molecular level in herbaceous model species. Our objective was to clone homologues of genes controlling the transition to flowering in tropical tree species. The *Arabidopsis* LEAFY (LFY) gene has been shown to be a key gene in the control of the transition to flowering, thus we have been concentrated on the cloning of LFY homologues from rubber tree (*Hevea brasiliensis*), mahogany (*Cedrela fissilis*), eucalypt (*Eucalyptus grandis*) and from a tropical pine (*Pinus caribaeae* var. *caribaeae*). Genomic libraries were constructed for each species and screened for LFY homologues using the *Arabidopsis* LFY sequence as a heterologous probe. Positive genomic clones were obtained from all four species and are being sequenced. Amino acid sequence comparisons have shown similarities ranging from 75 to 80% at the C-terminal end in relation to the *Arabidopsis* LFY protein sequence. Gene expression patterns, accessed by in situ hybridizations, indicated that the transcription of the cloned LFY homologues is correlated to the transition to the reproductive phase in the studied species. Developing efficient transformation protocols for these species would be the next step necessary for the manipulation of early-flowering genes in tropical trees. Acknowledgment: FAPESP

P-1130

Patterns of Storage Protein and Triacyl Glycerol Accumulation During Loblolly Pine Somatic Embryo Maturation. D. J. GIFFORD, S. L. Stone and C. D. Todd. Department of Biological Sciences, University of Alberta, Edmonton, Alberta, Canada, T6G 2E9. E-mail: DAVID.GIFFORD@UALBERTA.CA

Loblolly pine (*Pinus taeda* L.) somatic embryos synthesized and accumulated a set of seed storage proteins that were very similar to their zygotic counterparts. SDS-PAGE analysis identified three major proteins; a 60 KDa glutelin-like protein, comprised of 37.5 and 22.5 peptides linked by a disulphide bridge, a 47 KDa globulin, and a 15 KDa globulin. Accumulation of the 60 KDa and 47 KDa storage proteins was evident during early maturation; five weeks after transfer from liquid culture to maturation media; the 15 KDa globulin was not evident until mid maturation, nine weeks after transfer. Quantitatively, total embryo protein accumulation began two weeks after transfer, and maximum levels were attained six weeks later during mid maturation. On a fresh weight basis, anatomically mature somatic embryos contained approximately 44% of the total insoluble proteins and approximately 257% of the total soluble proteins found in their zygotic counterparts. Temporally, total triacyl glycerol (TAG) accumulation mirrored protein accumulation. On a fresh weight basis, anatomically mature somatic embryos contained approximately 27% of the total TAGs found in their zygotic counterparts.

P-1131

Fine Mapping and Development of SCAR Markers Linked to a Genomic Region Associated with Crown Form in *Cupressus sempervirens* L. N. HAMAMOUCH, P. Aravanopoulos, and A. Doulis. Dept. of Environment and Renewable Resources. Mediterranean Agronomic Institute of Chania, Crete, Greece. Email: andreas.doulis@nagref-her.gr

Despite the economic importance of crown shape and branching angle in tree species very little is known about the genetic control of these traits. Furthermore, none of the gene (s) controlling these traits have been identified or mapped. We used RAPD markers to fine map a genomic region linked with crown shape in the Mediterranean conifer *Cupressus sempervirens* L. that appears in two distinct forms (*C. sempervirens* var. *horizontalis* and *C. sempervirens* var. *pyramidalis*). Four RAPD markers linked with the genomic region controlling crown shape were cloned, fully sequenced and converted to SCAR markers. A total of eight SCAR primer pairs, with lengths ranging between 17 and 25 nucleotides were designed and custom synthesised. For all tests, the parents and 55 F_1 progeny trees were used. The male parent exhibited the pyramidal crown trait while the female parent exhibited the horizontal crown trait. The trait segregated 1:1 in the progeny. All SCAR primer pairs amplified bands of expected sizes. Nevertheless, only two primer pairs, SC-D05-I and SC-D09-I, amplified polymorphic bands between the parents. SCAR markers $SC\text{-}D05_{445}$ and $SC\text{-}D09_{640}$ maintained linkage with the appropriate phenotype but at lesser strength when compared to their original RAPD markers. The rest of SCAR primer pairs did not allow for differentiation between the parents and were not evaluated further.

P-1132

Photoautotrophic Micropropagation of Potential Energy Crops *Paulownia fortunei* and *Eucalyptus tereticornis*. P. S. SHA VALLI KHAN, T. Kozai, Q. T. Nguyen, C. Kubota, and V. Dhawan. Plant Tissue Culture, Biotechnology and Bioresources Division, Tata Energy Research Institute, Lodhi Road-110 003, New Delhi, India.

Fast growing woody crops can be used to produce high and consistent yield of biomass as fuel to generate electricity or make paper or other fiber products. The benefits in terms of reduced greenhouse gas emissions (CO2) are expected from both the sequestration of carbon on site as well as the replacement of non-renewable fossil fuels by a renewable energy source. A large number of studies carried out by different organizations have shown that tree energy crops could become significant components of the agricultural landscape in the future and require billions of high quality transplants every year for plantation programs. Current research aims for the first time to induce photoautotrophy in potential energy crops of industrial and ecological value like Paulownia fortunei, and Eucalyptus tereticornis, as alternative to conventional propagation and micropropagation systems. Photoautotrophic micropropagation provides several advantages over conventional micropropagation in achieving enhanced growth of the plantlets, lower contamination levels, reduced dependence on exogenous growth regulators, promotion of rapid, vigorous plant growth and development during the acclimatization stage. The growth, stomatal index, water relations and net photosynthetic rates of shoot cultures grown under various photoautotrophic conditions (without sucrose in the nutrient medium and with enriched CO2 and high photosynthetic photon flux or PPF) were compared with those of shoot cultures grown under conventional photomixotrophic conditions (30 gl-1 sucrose and 0.3 mgl-1 BA). In P. fortunei a higher sprouting percentage (100%) and more number of shoots (1.6) were obtained under conditions of high PPF and high CO2 concentration. The developed shoots (84%) were also rooted with an average of 5.9 roots per shoot with 4.01 cm length. Rooting of photomixotrophic shoot cultures was stimulated by a separate auxin treatment. The percent of moisture loss from leaves of photomixotrophic cultures was also varied than that of various photoautotrophic cultures. This is true at each of 30, 60, 90 and 120-minute intervals of air-drying. Leaves from photomixotrophic cultures had also higher stomatal index values compared to photoautotrophic cultures. The photoautotrophic growth with satisfactory net photosynthetic rate (NPR) was obtained in shoot cultures of E. tereticornis under conditions of high PPF and high CO2 concentration and using Florialite as a supporting material. None of the photoautotrophic cultural conditions resulted in the production of roots. Further research is in progress on the photoautotrophic culture conditions such as CO2 concentration, PPF, temperature, relative humidity, supporting materials etc. to produce high quality plantlets with a high growth rate, high NPR and large root system and commercialize photoautotrophic micropropagation system for these potential energy crops.

P-1133

Characterization of Transgenic Poplar with Ectopic Expression of Pine Cytosolic Glutamine Synthetase Under Varying Nitrogen Conditions. Hui-min Man, Randall Boriel, Rami El-Khatib, and EDWARD G. KIRBY. Department of Biological Sciences, Rutgers University, University Heights, Newark, NJ 07102. E-mail: ekirby@andromeda.rutgers.edu

We previously reported that ectopic expression of a pine cytosolic glutamine synthetase (GS1) gene in transgenic poplar resulted in significant alterations of biochemistry, early growth and development. In order to investigate the efficiency of nitrogen utilization in transgenic lines, young poplar GS transgenics and controls were grown under low nitrate (0.3 mM) and high nitrate (10.0 mM) conditions. Growth under low nitrate, as measured by average leaf area, total leaf area, total leaf numbers and average plant height, was significantly increased in transgenics as compared to controls. Under high nitrate (10.0 mM), growth of transgenics showed only slight increases over controls. Total GS activity in transgenics grown under low nitrate was approximately 60% higher (17.2 nkat gFW^{-1}) than in controls, and 40–50% higher (25.0 nkat gFW^{-1}) under high nitrate conditions. At both low and high nitrate conditions, average free leaf glutamine levels of transgenics were higher than in controls. However, glutamine levels in transgenics grown under high nitrate conditions were about two times higher than in controls. These results of the effects of manipulating nitrogen metabolism on intrinsic nitrogen utilization efficiency will be discussed in light of our recent work indicating that ectopic expression of the pine GS1 in poplar results in enhanced resistance to water stress.

P-1134

Mercuric Ion Reductase: An Alternative to Antibiotic Selection for Production of Transgenic Southern Pines. S. A. MERKLE, D. R. Smith, G. Andrade, P. M. Montello, and R. B. Meagher. Daniel B. Warnell School of Forest Resources, University of Georgia, Athens, GA 30602. E-mail: smerkle@uga.edu

Public concern regarding the use of antibiotic resistance genes as selectable markers for transformation of agronomic and forest species has prompted research aimed at developing alternative selectable markers. Heavy metal resistance genes, which our lab has been investigating for phytoremediation purposes, may also be useful as selectable markers. A modified bacterial mercuric ion reductase gene (*merA*), driven by a maize ubiquitin promoter, was transferred to embryogenic slash, loblolly and radiata pine cells via microprojectile bombardment. Cells were selected on EDM6 medium containing 15 micromolar mercuric chloride, followed by transfer of resistant colonies to 30 micromolar mercuric chloride. Resistant colonies appeared on the selection medium within as little as 10 days. In one experiment involving 17 embryogenic clones representing 4 mother trees, an average of 26 mercuric ion resistant transclones per gram of bombarded tissue was generated. PCR results followed by Southern hybridization of PCR signals with *merA* probe indicated that the mercuric ion resistant colonies contained the *merA* gene. Somatic seedlings from 6 independent transformed lines of slash pine have been regenerated. The mercuric ion reductase gene and mercuric ion selection may provide a useful alternative to selection with antibiotic or herbicide resistance genes and may result in more rapid capture of transgenic cultures as well.

P-1135

Genetic Transformation of Japanese Trees. TAKESHI MOHRI, Tomohito Igasaki, and Kenji Shinohara. Department of Molecular and Cell Biology, Forestry and Forest Products Research Institute, Ibaraki 305–8687, Japan. E-mail: mohri@ffpri.affrc.go.jp

Genetic engineering has the potential to allow the selective improvement of a single trait in forest trees without the loss of any of the desired traits of parental line. Using such techniques, we can overcome the difficulties associated with the breeding of long-lived perennials, which need a long time to produce progeny. Genetically transformed lombardy poplar (Populus nigra var. italica Koehne), Japanese white birch (Betula platyphylla var. japonica), black locust (Robinia pseudoacacia) plants were regenerated by co-cultivating tissue segments with Agrobacterium tumefaciens strain LBA4404 or GV3101 harboring a binary vector which contained the ß-glucuronidase (GUS) gene and neomycin phosphotransferase II or hygromaycin phosphotransferase gene. Successful transformation was confirmed by the ability of tissue segments to produce calli in presence of kanamycin or hygromycin, by histochemical and fluorometric assays of GUS activity in plant tisuues, and by Southern blot analysis. We also induced morphological abnormalities in lombardy poplar and black locust by engineering the overexpression of the rice homeobox gene OSH1. Thus, we established a simple and reliable procedure for the regeneration of transgenic Japanese broad-leaved trees. However, no studies of the transformation of Japanese conifers have been reported. We established a method for the microprojectile-mediated transfer of DNA and the transient expression of genes for GUS and luciferase in zygotic embryos of three species of Japanese conifer (Cyptomeria japonica D. Don, Pinus thunbergii Parl. and P. densiflora Sieb.), and a reproducible system for regeneration of plants from embryogenic callus of C. japonica. We have tried to develop the microprojectile-mediated transformation of C. japonica.

P-1136

Poplar Cell Suspension Cutures as a Tool to Study Wood Formation. ANNA B. OHLSSON, Kristina Blomqvist, Henrik Aspeborg, Li Xinguo, Anders Andersson, Hongbin Henriksson, Peter Nilsson, Tuula T. Teeri, and Torkel Berglund. Dept. of Biotechnology, KTH—Royal Institute of Technology, SCFAB, SE-106 91 Stockholm, Sweden. E-mail: annao@biochem.kth.se

Plant cell/tissue cultures constitute a convenient and rapid system to study plant biochemistry and molecular biology; especially in slowly growing species like trees. We have set up a cell/tissue culture system of the hybrid aspen *Populus tremula x tremuloides* as a model system to study selected aspects of wood formation. Starting from a poplar plantlet, we have established undifferentiated (fine and granular), root and shoot cultures in liquid medium. The cultures have been characterized with regard to wood related genes and enzymes during their growth cycle. Enzyme activities and gene expression related to primary cell wall formation, e.g., expansin, cellulase and XET (xyloglucan-endo-transglycosylase), occur primarily during the mid-logarithmic part of the growth curve. In contrast, e.g., laccase and peroxidase, related to lignification and secondary cell wall formation, showed maximum activity during the stationary phase. Induction studies of the cultures are presently beeing performed using different hormones and growth factors. The poplar cell cultures can also be used for small scale production of native plant proteins for comparative studies with the corresponding heterologous proteins. For example we have purified active XET from the shake flask grown fine suspension cultures.

P-1137

Cryopreservation of *Populus maximowicz* Henry Seeds. JAE-IN PARK and Nak-Sun Kim. School of Forest Resources, Chungbuk National University, Cheongju 361–763 Republic of Korea. Email: jipark@cbucc.chungbuk.ac.kr

To establish a cryopreservation method of *Populus maximowicz* Henry germination of seeds was tested after stored 1 hour, 1day, 5 days, 1 to 5 months and 7 months respectively at 3 levels of storage temperature; room temperature, cold storage (4°) in a refrigerator, and ultra low temperature(-196°) in liquid nitrogen. Shortly after ripening the seeds were collected and subjected to test. Three replications of 20 seeds were used for each treatment. Germination rate was calculated by counting germinated seeds on wet filter paper in petri dishes at 25° At room temperature the germination rate dropped abruptly to 0% within a month, and at 4° it did gradually whereas at ultra low temperature it maintained the same rate as initial one. This means that the seeds of *Populus maximowicz* can be stored by cryopreservation.

P-1138

Brassinolide Improves Embryogenic Tissue Initiation in Conifers. G. S. PULLMAN and G. F. Peter. Institute of Paper Science and Technology, Atlanta, GA 30318. E-mail: jerry.pullman@ipst.edu

Somatic embryogenesis (SE), the most promising technology to multiply high-value coniferous trees from advanced breeding and genetic engineering programs, is expected to play an important role in increasing productivity, sustainability, and uniformity of future U.S. forests. To be successful for commercial use, SE technology must work with a variety of genetically diverse trees. Initiation in loblolly pine (Pinus taeda L), our main focus species, is often recalcitrant for desirable genotypes. Initiation rates of loblolly pine, Douglas fir (Pseudotsuga menziesii), and Norway spruce (Picea abies) were improved through the use of Brassinolide. Brassinolide is a brassinosteroid found in many plant species. Brassinosteroids are a group of naturally occurring steroidal lactones that include brassinolide and its analogs. The brassinosteroids were relatively recently discovered plant growth regulators. In angiosperm species, brassinosteroids have been shown to have diverse, tissue-specific, and species-specific effects, including the stimulation of cell elongation, stimulation of ethylene production and increasing resistance to abiotic stress. In our media, brassinolide was effective at concentrations ranging from 0.005–0.25 mM. Using control media (no brassinolide) and 0.1mM Brassinolide, initiation rates in loblolly pine, Douglas fir, and Norway spruce were improved from 15.0 to 30.1%, 16.1 to 36.3%, and 34.6 to 47.4% respectively. Initiation rates in loblolly pine were also improved through the combination of modified 1/2 P6 Salts, activated carbon at 50 mg/l, Cu and Zn adjusted to compensate for adsorption by activated carbon, 1.5% maltose, 2% myo-inositol (to raise osmotic level partially simulating the ovule environment), 500 mg/l case amino acids, 450 mg/l glutamine, 2 mg/l NAA, 0.63 mg/l BAP, 0.61 mg/l kinetin, 3.4 mg/l silver nitrate, 10mM cGMP, 0.1 mM brassinolide, and 2 g/l Gelrite. Across 12 open-pollinated families of loblolly pine, initiation rates ranged from 2.5–50.7% averaging 22.5%.

P-1139

Cell Engineering of Japanese Conifers, *Cryptomeria japonica* and *Larix kaempferi*. HAMAKO SASAMOTO[1], Shinjiro Ogita[2], Mitsue Fukui[3]. [1]Faculty of Environment & Information Sciences, Yokohama National Univ.-Yokohama 240–8501; [2]Research Association for Biotechnology, NAIST-Nara 630–0101; and [3]Forestry and Forest Products Res. Inst.-Tsukuba 305–8687, JAPAN. E-mail: sasamoto@ynu.ac.jp

Regulatory factors for somatic embryogenesis of Japanese conifers were investigated, and also several cell engineering techniques were developed. Embryogenic cultures of Japanese conifers, *Cryptomeria japonica* and *Larix kaempferi*, were induced and maintained for long periods in liquid culture. Both exogenous application of auxin, cytokinin, glutamine, and endogenous levels of gibberellins, abscisic acid, and amino acids, were found to be important factors for the cultures. Furthermore, cell density was a notable key factor for the maintenance of the cultures and maturation of somatic embryos from them. Our data from the cell density manipulation in liquid culture clearly showed the necessity to optimize the culture conditions in relation to embryogenic cell morphology. We succeeded in recovering of embryogenic cells in liquid suspension cultures from protoplasts. Optimal combinations of cell wall-degrading enzymes differed during developmental stages of embryogenic tissues. The optimal basic media for *Cryptomeria* and *Larix* protoplasts were MS and NH4NO3-free MS, respectively. Using liquid media of small-volume, e.g. 50 ll, in a multi-well plastic culture plate, a quick survey of different concentrations of regulatory factors is possible. Single protoplast-derived tissue structure was selected using a micromanipulator and separately cultured in a 96-well culture plate, and developed into embryogenic suspension cultures. Mature somatic embryos were formed when ABA was added in maturation media. Using the above culture system, it is possible to microinject GFP-gene or GFP itself and to detect the specific fluorescence.

P-1140

Establish the Gene Transgenic System of *Paulownia fortunei*. YA-NAN WANG, Kai-Wum Yeh, and Shei-Chun Han. National Taiwan University, Forestry Department, No. 1, Sect. 4, Rosevolt Road, Taipei, Taiwan 106. Email: m627@ccms.ntu.edu.tw

We introduced the insect resistant gene-sweet potato sporamin to LBA4404 of *Agrobacterium tumefaciens* with a binary vector PBI121. We then transferred the gene protein of sporamin to *P. fortunei* with CaMV35S as the promoter and the trypsin inhibitor was expressed. We used the medium with 30 ppm antibiotic kanamycin as the first selection marker to screen out the successful transgenic plants. Then we checked the responses of the transgenic plants with PCR to reconfirm the genomic DNA of the transgenic *P. forrtunei* with fragment of sporamin. We used southern blot analysis to test the transgenic plants selected from the PCR and could see clear signals on the X-ray film. We used northern blot to test the transgenic plants and found out the fragment of sporamin could be expressed on mRNA. At last, we detected the protein activity of sporamin in TP1, TP2, TP5 and TP6 with the trypsin inhibitor activity staining method. We also had the same results from western blot method. Therefore we concluded that the transgenic plants could steadily express sporamin, but it was possible for the TP3 and TP4 to express gene silencing.

P-1141

Produce and Characterize Several Classes of Plantibodies (Plant Made Monoclonal Antibodies). YONG-QIANG (CHARELS) AN, JiPing Yao, Jesus Velasco, Rosa Carcamo, Natasha Bohorova, Greta Chillag, and Andy Hiatt. Epicyte Pharmaceutical, Inc., 5810 Nancy Ridge Drive, Suite 150, San Diego, CA 92121. Email: yqan@epicyte.com

Currently, 25% of biopharmaceuticals under development are monoclonal antibodies. High cost to manufacture antibodies in mammalian cells and needs for a large amount of antibodies limits the economic application of antibodies to disease prevention. However, Plants can produce and assemble monoclonal antibodies and allow producing an unlimited amount of antibodies (plantibodies) at low cost. Epicyte is developing plantibodies to prevent infective diseases such as HIV and HSV (herpes simplex virus) and RSV (respiratory syncytial virus). Herpes is an incurable sexually transmitted disease caused by HSV. It is estimated that 1 in 5 adults (50 million) in the US are infected with genital herpes. One million new infections occur each year. Preventing HSV infection is paramount. Vaginal delivery of an anti-HSV monoclonal antibody and plantibody has been shown to provide complete protection against genital herpes in a mouse model. HSV8 is a human monoclonal antibody. It binds to glycoprotein D, a well-characterized antigen. In the presentation, using production of HSV8 plantibody as an example, we will discuss producing anti-HSV IgG, IgA, dIgA and sIgA in plants, including engineering antibody genes, screening transgenic plants for plantibody expression and assembly, and plantibody purification and characterization. Producing new forms of plantibodies and the technical challenges to produce the plantibodies will be discussed as well.

P-1142

The Conservation of a Vital European Scientific & Biotechnological Resource: MicroAlgae & Cyanobacteria: "COBRA". E. BENSON, J. Elster, J. Lukavsky, A. Lukesova, T. Friedle, M. Lorenze, M. Herdman, R. Rippka, H. Hedoin, T. Hall, L. Santos, F. Santos, J. Day, D. Bremner, L Natanson, and S. Watt. University of Abertay Dundee, Department of Science and Engineering, Plant Conservation Group, Bell Street, Dundee Tayside, DD11 HG, United Kingdom. www.cobra.ac.uk. E-Mail: jgd@ceh.ac.uk.

COBRA is the acronym for a European Union, Research Infrastructures Project (No. QLRT-2000–01645). It has a remit to develop a physical and virtual European Biological Resource Center for algal culture collections and apply cryopreservation to currently "preservation recalcitrant" strains of microalgae and cyanobacteria. These organisms are difficult to conserve using current cryogenic methods. Molecular and biochemical stability tests will be developed to ensure that equivalent strains supplied by European culture collections will give high quality and consistent performance. Stress physiology research is a component of the project and will assist the optimization of methods for preserving a wide range of algal diversity. COBRA's "Resource Centre" will utilize Information Technologies (IT) and Knowledge Management to assist project coordination, management and information dissemination. This will also facilitate new knowledge creation pertaining to algal conservation. This project will, for the first time, in Europe, utilize cryopreservation as a standard technique for algal conservation, thereby ensuring the genetic stability of conserved strains. The European biotechnology sector will be provided with the organisms they require, as well as a guarantee of their long-term availability and stability. COBRA partners are from different sectors (Culture Collections, Biotechnology Industries and Academia) and countries (Czech Republic, France, Germany, Portugal, UK).

P-1143

Transgenic Plant Cells Express Anti-infective Monoclonal Antibodies. N. BOHOROVA, R. Carcamo, J. Velasco, J. Yao, Y. Q. An, G. Chillag, A. Hiatt. Epicyte Pharmaceutical, Inc., San Diego, CA 92121. E-mail: nbohorova@epicyte.com

Transgenic plants are attractive and cost-effective alternatives for the production of biomolecules, because large amounts of antibodies can be produced at relatively low cost, using agriculture instead of expensive mammalian cell culture-based expression systems. Many infectious agents such as bacteria and viruses that are transmitted via contaminated food, water or sexual contact, colonize and invade epithelial membranes. Immunoglobulins, such as IgA, IgG, IgM and IgG/IgA chimeric antibodies are effective against these infections by stimulating the mucosal immune system. By introducing specific human antibody genes into plants, plant cells can be reprogrammed to manufacture human proteins capable of producing human antibodies or "plantibodies" (plant + antibodies). Other proteins needed to produce new pharmaceutical drugs and diagnostics can also be synthesized with this approach. We have been able to produce plant material stably transformed with full-length human antibodies: Respiratory Syncytial Virus (RSV) and Herpes Simplex Virus (HSV). Development of an effective plant transformation system for expressing monomeric human antibodies and correct assembly of complex immunoglobulin molecules in plant cells will be discussed.

P-1144

'Bio Plastics' in Transgenic Plants: Cyanophycin—a Suitable Source for Polyaspartat. INGE BROER[1], Katrin Neumann[1], Wolfgang Ziegler[2]*, and Wolfgang Lockau[2]. [1]Universität Rostock, Justus v. Liebigweg 8; D-18051 Rostock and [2]Humboldt Universität Berlin, Chaussestr. 117; D-10115 Berlin *Bayer AG Leverkusen. E-mail: Inge.Broer@biologie.uni-rostock.de

The production of biodegradable polymers, which will be used to substitute petrochemical compounds in commercial products, in transgenic plants is an important challenge for plant biotechnology. The genes necessary for the expression of polyhydroxybutyrate, a solid polymer, have successfully been transferred to arabidopsis and sugar beet. The production of the liquid bio plastic polyaspartat, which is already used to substitute Polycarboxylat, in transgenic potato tubers, is the topic of our project. Polyaspartat is the main component of the bacterial storage protein Cyanophycin, a polymer composed of L-Aspartat and L-Arginin. Cyanophycin is produced via non-ribosomal protein biosynthesis by Cyanophycin synthetase. Three genes coding for different Cyanophycin synthetases have been isolated form Synecocystis, Anabena and Synechococcus strains and their coding regions have been fused to constitutive and tuber specific promoters. By the addition of a signal peptide to the N-terminus of the proteins, the enzymes have been transferred to plant chloroplasts. Although the survival of transgenic plants did depend on the gene used and seems to be enhanced in transformands producing Cyanophycin in the chloroplast, we could prove, that sufficient amounts of the protein can also be synthesized in the cytoplasm of plants constitutively expressing a Cyanophycin synthetase. We are currently enhancing Cyanophycin production in potato tubers.

P-1145

Phosphorylation of Tristetraprolin by Mitogen-activated Protein Kinases. HEPING CAO, Ester Carballo, Frederick Dzineku, Wi S. Lai, Elizabeth A. Kennington, and Perry J. Blackshear. Office of Clinical Research and Laboratory of Signal Transduction, National Institute of Environmental Health Sciences, Research Triangle Park, NC 27709. E-mail: cao2@niehs.nih.gov

Tristetraprolin (TTP) and its related proteins are characterized by two $CX_8CX_5CX_3H$ zinc finger binding domains separated by 18 amino acids. Similar sequences have been found in at least 14 species including rice and *Arabidopsis*. TTP is an mRNA-binding protein that promotes the destabilization of certain mRNAs containing class II AU-rich elements. However, it has been difficult to purify TTP for biochemical studies and antibody production. We expressed active recombinant human TTP (hTTP) in *E. coli* using a maltose-binding protein (MBP) system. MBP-hTTP was purified to apparent homogeneity by amylose resin, Superose 12, and Mono Q columns. TTP cleaved from purified MBP-hTTP with PreScission protease precipitated out of solution but could be purified to homogeneity by continuous-elution electrophoresis. The identity of MBP-hTTP and hTTP was confirmed by N-terminal sequencing and matrix-assisted laser desorption ionization mass spectrometry analysis. Both MBP-hTTP and hTTP bound to tumor necrosis factor alpha (TNF-alpha) mRNA in an electrophoretic mobility shift assay. However, MBP-hTTP purified by a Mono Q column in a buffer containing EDTA did not bind to the TNF-alpha mRNA, whereas Zn^{++} in the binding buffer in excess of EDTA partially restored its mRNA-binding activity. MBP-hTTP separated into two broad protein peaks on Sephacryl S200 gel filtration. Fractions eluting earlier than those corresponding to the monomer size exhibited mRNA-binding activity, suggesting that a dimer or polymer forms the active species. MBP-hTTP, but not MBP, was phosphorylated in vitro by mitogen-activated protein (MAP) kinases including p42, p38, and C-Jun N-terminal kinases. Similar results were obtained using an MBP fusion protein with mouse TTP. This study demonstrates that TTP's RNA binding activity is zinc-dependent, and that TTP can be phosphorylated by JNK and other MAP kinases.

P-1146

Maize Derived LT-B Induces a Strong Immunogenic Response in and Protects BALB/c Mice Against *Escherichia coli* Heat Labile Enterotoxin (LT) And Cholera Toxin (CT). RACHEL CHIKWAMBA, Joan Cunnick, Diane Hathaway, Jennifer McMurray, Hugh Mason, and Kan Wang. Iowa State University, Department of Agronomy, Plant Transformation Facility, B538 Agronomy Hall, Ames, IA 50011. E-mail: rchikwam@iastate.edu

Plant transformation technologies have facilitated the production in plants of novel proteins. Transgenic plants offer the potential to be one of the most economical systems for large scale production of high value proteins for industrial, pharmaceutical, veterinary and agricultural use. We have generated transgenic maize expressing a synthetic gene encoding the B sub-unit of the *Escherischia coli* heat labile enterotoxin (LT-B). LT-B is an ideal model protein for an oral vaccine, inducing a strong serum and mucosal response upon oral administration. Mucosal immunity plays a critical role in protection from effects of the enterotoxin. LT-B is also a strong mucosal adjuvant, with potential to enhance the value of other orally administered vaccines. Maize is a major component of livestock feed, and therefore an attractive crop for the production and delivery of an oral vaccine. We demonstrate in this study that the maize generated LT-B is functional, with physical and biochemical properties of the native LT-B such as pentameric structure, ganglioside binding capacity and immunogenicity in mice. Maize generated LT-B generated a significantly stronger serum and mucosal immune responses in BALB/c mice than an equivalent amount of the bacterial derived LT-B. Delivery of LT-B within seed tissues appears to have important implications on the exposure of antigen to the gut associated lymphoid tissues (GALT) and the extent of the resultant immune response. When challenged with enterotoxigenic heat labile and cholera toxins (LT and CT), orally immunized BALB/c mice were protected from effects of these toxins. These results demonstrate the potential of corn as a production and delivery system for oral vaccines.

P-1147

Modification of Anthocyanin Biosynthesis in Potato (*Solanum tuberosum*) Cell Cultures. S. C. DEROLES, K. E. Schwinn, D. H. Lewis, J. M. Javellana, C. E. Lee. Crop & Food Research, Private bag 11600, Palmerston North, 5301, New Zealand. E-mail: deroless@crop.cri.nz

Anthocyanins and flavonoids are natural plant pigments present in almost all fruit and vegetables that provide a variety of health benefits such as reduced heart disease and cancer rates. Because of these health benefits, anthocyanins are now sought after for use as natural food colourants in processed food to replace coal tar based artificial dyes. Currently the primary source of anthocyanin pigments for food colourants is from grape skins, a by-product of the wine industry. An alterantive source is production via plant cell cultures in large bioreactors. However, currently there are no cell cultures able to produce anthocyanins at a commercially viable yield, although there is much research to obtain such a line. The use of plant cell cultures will enable the production of a consistent supply of anthocyanins and other flavonoiod compounds known to have health benefits (nutraceuticals). They would also enable greater quality control and, through the use of genetic modification, the production of specific compounds suitable to the food processing industry. We have isolated a potato (*Solanum tuberosum*) cell line that is able to produce high amounts of anthocyanin in the dark. However the cell line requires constant selection of coloured cells and has a slow growth rate when compared to non-coloured isogenic lines. The onset of anthocyanin production in this line seems to be associated with a change in the differentiation state, possibly the first stage in embryogenesis. We have developed a transformation system for our potato cell cultures to study the control of anthocyanin biosynthesis in undifferentiated cell lines.

P-1148

Expression of Human Proinsulin in Transgenic Chloroplasts. ANDREW L. DEVINE, Peter O. Wiebe, and Henry Daniell. Department of Molecular Biology and Microbiology, University of Central Florida, 12722 Research Parkway, Orlando, FL 32826-3227. Email:daniell@mail.ucf.edu

Oral administration of disease-specific autoantigens can prevent or delay the onset of autoimmune disease symptoms. We are generating tobacco and tomato chloroplast transgenic plants that synthesize human proinsulin, a major insulin-dependent diabetes mellitus autoantigen. Hyper-expression of foreign proteins (up to 46% of total soluble protein) has been accomplished in our lab via chloroplast genetic engineering. Hyper expression of proinsulin in tobacco can lead to a cheaper and more efficient process for purification. For example, 60% of the total operating cost in the production of human insulin is associated with *in vitro* processing (formation of disulfide bridges). In purification of insulin, chromatography accounts for 30% of operating expenses and 70% of equipment in production. To direct delivery of plant-synthesized insulin to the gut-associated lymphoid tissues, proinsulin is fused to the C-terminus of the cholera toxin beta subunit (CTB). Previously, we generated chloroplast transgenic plants expressing CTB with proper disulfide bridges that resulted in the accumulation of up to 4.1 % of total soluble tobacco leaf protein as functional oligomers. Here, we investigate several avenues for optimal gene expression. We have synthesized the proinsulin gene that has a codon composition consistent with elevated chloroplast gene expression, and we will compare expression with the native proinsulin gene. Additionally, we have generated constructs with differing 5' untranslated regions, and will measure the differences in their expression. We will also characterize each transgenic line for expression of functional pentameric CTB-proinsulin fusion by measuring GM1-ganglioside binding affinity. Ultimately, we will use the transgenic tomato chloroplast plant with the greatest functional expression to test the effect on the progression of clinical diabetes on nonobese diabetic mice.

P-1149

Efficient Chloroplast Transformation of Tomato with an Edible Selectable Marker. AMIT DHINGRA, Seung Bum Lee, and Henry Daniell. Department of Molecular Biology and Microbiology, University of Central Florida, 12722 Research Parkway, Orlando, FL 32826–3227. Email: daniell@mail.ucf.edu

The technology of chloroplast transformation holds great promise for the future of plant biotechnology. It allows for single step multigene engineering, high expression of the introduced gene without the drawbacks of gene silencing and position effect. Maternal inheritance of the chloroplasts ensures gene containment thus making it environmentally friendly. We have established a rapid protocol for engineering the tomato chloroplast genome by employing the spinach derived Betaine Aldehyde Dehydrogenase gene. The tomato chloroplast transformation vector employed in the study integrates the transgene into the transcriptionally active spacer region of *trnI*/*trn*A in the inverted repeat region via homologous recombination. On the selection media, primary shoots were obtained within three weeks as compared to over a year with *aad*A gene as the selectable marker. Chloroplast specific integration of the transgene has been confirmed by PCR. Further, homoplasmy will be confirmed by Southern analysis. Tomato chromoplasts will be used to express therapeutically important proteins like cholera toxin beta subunit and anthrax protective antigen under the control of upstream regulatory sequences. Towards this end, T7 gene10 and *cry*2Aa 5' untranslated regions will be used in order to enhance expression in tomato fruit chromoplasts. This would facilitate oral delivery of these proteins thereby paving the way for production of edible vaccines. Most importantly, the utilization of an edible plant-derived selectable marker would help in allaying public concerns about GM foods.

P-1150

Expression of Interferon alpha2b in Transgenic Chloroplasts of an Edible Tobacco for Oral Delivery. REGINA A. FALCONER and Henry Daniell. Department of Molecular Biology and Microbiology, University of Central Florida, 12722 Research Parkway, Orlando, FL 32826. Email: daniell@mail.ucf.edu

Currently, recombinant IFNa 2b is being used for the treatment of viral and malignant diseases. More recently, IFNa 2b has been used in conjunction with other drugs resulting in a synergistic boost to the treatment. However, up to 20% of patients develop anti-IFNa antibodies which may be related to route administration, dosage parameters, or production based contaminants. Oral delivery of IFNa 2b expressed via the chloroplast genome may eliminate these side effects. In fact, oral administration of natural human IFNa has been shown to elicit a systemic immune response. We have generated a recombinant IFNa 2b construct containing a polyhistidine purification tag as well as a thrombin cleavage site. Already, this construct has been integrated into the chloroplast genome of a low nicotine variety of tobacco, LAMD-605. Ultimately, we will analyze protein expression with northerns, westerns, and ELISA. Chloroplast transformation has led to hyperexpression of foreign proteins due to an high ploidy number (up to 10,000 copies of transgene per cell). Chloroplasts can correctly process and fold foreign proteins as well as form the requisite disulfide bonds. However, previous studies in our lab have shown that interferons may be degraded in the chloroplast. To address this problem, we have generated another construct to express a fusion protein with the chloroplast friendly green fluorescence protein (GFP). The GFP/IFNa 2b fusion with a contiguous furin cut site will provide protection from degradation, facilitate purification, and leave the mature IFNa 2b in the intestines of mice for future animal studies. Results of these investigations will be presented.

P-1151

Manipulation of Gene Regulation in Transgenic Tobacco Chloroplasts Results in Hyper-expression of Human Serum Albumin, Formation of Inclusion Bodies and Facilitates Purification. [1]A. FERNÁNDEZ-SAN MILLÁN, [2]A. M. Mingo-Castel, [3]M. Miller, and [1]H. Daniell. [1]Department of Molecular Biology & Microbiology and Center for Discovery of Drugs and Diagnostics, University of Central Florida, Orlando, FL 32826; [2]Instituto de Agrobiotecnología y Recursos Naturales-CSIC, Public University of Navarra, Mutilva Baja, 31192 Navarra, Spain; and [3]Auburn University Research Instrumentation Facility—Advanced Microscopy and Imaging Laboratory, Auburn, AL 36849. E-mail: alicia_fern@yahoo.es

Human Serum Albumin (HSA) accounts for 60% of the total protein in blood serum and it is the most widely used intravenous protein. To date, HSA has been produced primarily by the fractionation of blood. In spite of screening the raw material and heat treatment of final product, HSA derived from blood might harbor pathogens. Recombinant DNA technology provides a way of overcoming such concerns. Plants may be a suitable alternative to microbial or animal expression of HSA because of their inexpensive production costs and lack of human pathogens. However, level of expression of HSA in nuclear transgenic plants has been disappointingly low. As an alternative approach, transgenic chloroplasts can accumulate large amounts of foreign proteins and have been shown to be able to properly fold several recombinant proteins and form disulfide bonds. Therefore, different vectors were constructed and bombarded into tobacco leaves to direct HSA expression in transgenic chloroplasts. Regulation of HSA under the control of a Shine-Dalgarno sequence (SD), 5' *psbA* region or the *Bacillus thuringiensis cry2Aa2* UTR resulted in different levels of expression in transgenic chloroplasts from *in vitro* seedlings: 0.8, 1.6, 5.9% of HSA in tsp, respectively. A maximum of 0.02, 0.8 and 7.2% of HSA in tsp was observed in transgenic potted plants regulated by SD, *cry2Aa2* UTR or 5' *psbA* region respectively, demonstrating excessive proteolytic degradation, unless compensated by enhanced translation. The *psbA*-HSA expression was subject to developmental and light regulations, with the lowest expression observed in *in vitro* seedlings and maximal expression (11.1% tsp) under continuous lighting. HSA accumulated into large inclusion bodies, however, this led to gross underestimation of HSA because ELISA could be performed only in partially solubilized plant extracts. In spite of this underestimation, we report here the highest expression of a pharmaceutical protein, achieving levels 500-fold higher than previous reports of HSA expression in nuclear transgenic plants. Formation of HSA inclusion bodies not only offered protection from proteolytic degradation but also provided a simple method of purification from other cellular proteins by centrifugation. HSA inclusion bodies could be readily solubilized to obtain monomeric form using appropriate reagents. The *cry2Aa2* UTR mediated expression in seedlings and chromoplasts, although as efficient as *psbA* 5' region, is independent of light regulation and should therefore facilitate expression of foreign genes in non-green tissues, thereby enabling oral delivery of pharmaceuticals.

P-1152

Production of Active Substances Applying Innovative Plant Biotechnology. ANDRÉ GERTH(1), Elio Jimenez Gonzales(2), Rafael Gomez Kosky(2), Dirk Wilken(1). (1) BioPlanta GmbH, Benndorfer Landstr. 2, 04509 Delitzsch, Germany and (2) Instituto de Biotecnología de las Plantas, Carretera a Camajuaní Km. 5 1/2, Santa Clara, Cuba. E-mail: andre.gerth@bioplanta-leipzig.de

Active substances obtained from plants are known for their complex and well-tolerated biological effects. The quality of active substances from plants harvested in nature or cultivated in fields is instable because it depends on the environmental conditions (weather, climate, seasons and soil). Infestation, deseases and the application of pesticides additionally decreases the quality of the active substances. According to this problems the objective of the work is to develop a technology for stable production of high quality active substances. For this purpose the development, optimization and application of innovative techniques for metabolite production was necessary. Applying the Temporary Immersion System, a ten fold increase of the biomass within three weeks can be reached. It was possible to change the spectrum of the active substances by controlling the cultivation conditions. Cell cultures were multiplied in bioreactors up to 500% within 6 days. A content of active substances up to 1% of the fresh weight was obtained. The result of the research is a base technology for biotechnical production of active plant substances especially for the pharmaceutical and cosmetic industry. The focus of this technology is on modern techniques to cultivate the whole plant, plant organs and cells in bioreactors and Temporary Immersion Systems. Advantages of the developed technique are high and stable quality of active substances, high multiplication rate of biomass with high content of active substances, no loss due to pests or diseases, the possibility to influence the spectrum of active substances by controlling all cultivation conditions and the possibility to produce genetically altered organisms without endangering the environment. With this technology active substances can be produced successfully in Temporary Immersion Systems with a higher yield and a higher quality than in the field.

P-1153

Expression and Immunogenicity of Rabies Glycoprotein Produced in Tobacco and Muskmelon Plants by *Agrobacterium* Mediated Transformation. P. H. RAMANJINI GOWDA[1], R. Shilpa[1], S. N. Madhusudana[2], A. N. Dineshup[1], C. S. Prakash[3], T. K. S. Gowda[1], B. N. Devaiah, and N. Nagesh. [1]Department of Biotechnology, UAS, GKVK, Bangalore – 560065, India; [2]Department of Neurovirology, NIMHANS, Bangalore-560029, India; and [3]Center for Plant Biotechnology, Tuskegee University, Tuskegee, AL. Email: ramanjini@yahoo.com

Use of genetically altered plants for production of vaccines is an upcoming concept gaining importance in recent days. In this study rabies glycoprotein was expressed in tobacco and Muskmelon plants and its immunogenicity was evaluated in experimental mice. The transgenic Tobacco and Muskmelon plants were obtained after cocultivation with rabies glycoprotein gene. PCR, dotblot, Native PAGE and westren blotting confirmed the expression of rabies glycoprotein in transgenic Tobacco and Muskmelon. The Tobacco and Muskmelon crude protein extracts were capable of inducing antibodies when inoculated in mice, which also resulted in protection from subsequent challenge with CVs strain of virus. These results clearly demonstrate that the present system of expression has resulted in formation of adequate quantities of rabies glycoprotein in Tobacco and Muskmelon plants. This is the first report that the rabies glycoprotein produced in plants can give protection to mice from rabies.

P-1154

Expression of an SIV Protein in Transgenic Maize for Use as an Edible Vaccine and Reagent Supply. MICHAEL E. HORN, Susan L. Woodard, Richard C. Clough, Susan M. Souers, and Joseph M. Jilka. ProdiGene, 101 Gateway Blvd., Suite 100, College Station, TX 77845. E-mail: mhorn@prodigene.com

There are several reports demonstrating that antigens derived from various pathogens can be synthesized at high levels in their authentic forms in plants. When administered orally by feeding, edible vaccines can induce an immune response and, in some cases, have shown to result in protection against a subsequent challenge with the pathogen. Storage and delivery of a traditional vaccine is an issue in developing countries due to problems such as lack of refrigeration. Many such problems can be alleviated using edible vaccines. Transgenic maize could be an excellent source of HIV-related proteins for edible vaccines as well as costly reagents. Toward these goals, we have transformed maize with an SIV protein gene. We have obtained expression of the protein in both transgenic callus and plants. Details of the system, including quantification of the protein in first generation seed, will be presented. NIH #1R21A1048374–01

P-1155

Expression of Synthetic Human Hemoglobin Genes in Transgenic Tobacco Chloroplasts. E. KELLY-WHITE, A. Dhingra, P. Wiebe, H. Daniell. Department of Molecular Biology and Microbiology, University of Central Florida, Orlando, FL 32829–3227. E-mail: daniell@mail.ucf.edu

With a critical need for donated blood and continued concern of the pathogenic contamination of the current supply, the need for alternative sources of blood and blood substitutes is evident. The expression of human hemoglobin has been described in transgenic tobacco previously via nuclear transformation targeted to the chloroplast in which expression levels were found to be quite low, 0.05% total extracted seed protein. We intend to achieve considerably higher levels of expression using chloroplast transformation. Hyper-expression of foreign proteins has been repeatedly achieved in our laboratory using this technique, as high as 47% total soluble protein. The two genes necessary for the synthesis of human hemoglobin include hemoglobin alpha and beta. These proteins assemble into a functional tetramer with the incorporation of a heme molecule. The gene sequences, of both alpha and beta, have been optimized for enhanced translation within the chloroplast. The expression levels of each of these synthetic genes will be further regulated with a selective organization of 5' and 3' untranslated regions. The assembly of the functional tetramer within the chloroplast will be accomplished with the introduction of heme, a natural bi-product of chlorophyll synthesis. Functionally we anticipate that synthetic human hemoglobin will have a similar affinity for oxygen, Bohr and phosphate effects as native human hemoglobin. It has been reported that these factors have not been successfully addressed in other attempts of recombinant hemoglobin due to the presence of the amino terminal methionine. Based on the amino acid sequence, the terminal methionine will be removed by the aminopeptidase activity naturally present in the chloroplast lending to proper function of synthetic hemoglobin.

P-1156

Transgenic Plants as a Source of "Edible Vaccine" for Two Morbilliviral Animal Diseases. ABHA KHANDELWAL, M. S. Shaila, and G. Lakshmi Sita. Department of Microbiology and Cell Biology, Indian Institute of Science, Bangalore, 560 012, INDIA. Email: abhak@mcbl.iisc.ernet.in

Two animal diseases, Rinderpest and *Peste des petits ruminants* (PPR) often known as "cattle plague" and "goat plague" respectively because of the fatal and contagious nature of the disease, are caused by negative stranded RNA viruses known as Rinderpest and Peste des petits ruminants virus. These enveloped viruses harbor two surface glycoproteins Hemagglutinin (H)/ Hemagglutinin-Neuraminidase (HN) and Fusion (F) protein which are highly immunogenic and protective. International vaccination programs are successful in limiting the distribution of disease but loose pockets of infection remain a threat for the world considering the devastating outbreaks of disease in the past which killed hundreds of millions of host animals and wild ruminants. The available vaccines are heat labile and need the cold chain to maintain the potency of vaccine. The recombinant subunit heat stable vaccines have been produced in vaccinia/capripox or in baculo viruses but field trials are still in progress. At this point, it is important to consider that the vaccine given orally could prove more effective means of immunization as most of the infectious agents come in contact with the host at the mucosal surfaces. To eradicate these diseases from the developing world, new generation vaccines which are cost effective and heat stable are required. The use of plants as a production system for the foreign proteins has opened a new era for production and delivery of vaccines. The transgenic plants expressing vaccine antigens can be used as a source of edible oral vaccine. With the aim of developing edible vaccines for rinderpest and PPR, we have generated transgenic peanut (*Arachis hypogaea* L.) plants expressing H /HN. The antigenic authenticity of the plant derived-H/HN is confirmed by Western blotting and ELISA using a series of antibodies including convalescent sera. The immunogenicity of the plant derived- H/HN have been studied in a mouse model and the protective ability of these antibodies have been tested *in vitro* by Virus Neutralization test. Cattle and sheep fed with transgenic peanut leaves expressing H/HN elicit high levels of antibodies reacting to H/HN. These antibodies are able to inhibit the binding of H/HN specific monoclonal antibodies in c-ELISA. Further, these antibodies are able to neutralize virus infectivity *in vitro*. The priming of T cells, which take part in cell mediated immune response, has been shown by *in vitro* lymphoproliferation assays. In summary, plant derived-H/HN when supplied orally as a part of food, are able to induce both arms of immune response. Thus, these results indicate the potential of a plant based edible vaccine for Rinderpest and *Peste des petits ruminants* virus.

P-1157

Involvement of Protein Phosphorylation in rol Gene-Dependent Activation of Anthraquinone Production in Transgenic Cell Cultures of *Rubia cordifolia*. V. P. Bulgakov, M. V. KHODAKOVSKAYA*, G. K. Chernoded, N. P. Mischenko**, and Yu. N. Zhuravlev. *Departmnent of Plant Sciences, University of Connecticut, Storrs, CT 06269; The Institute of Biology and Soil Sciences of the Far-East Branch of Russian Academy of Sciences, Vladivostok, 690022, Russia; and **Pacific Institute of Bioorganic Chemistry, Far-East Branch of Russian Academy of Sciences, Vladivostok 690022, Russia. E-mail: m_khod@yahoo.com

It has been suggested that the *rol* genes of *Agrobacterium rhizogenes* could play an essential role in the activation of secondary metabolite production in plant transformed cultures. This study investigated whether the content of anthraquinone phytoalexins was changed in callus cultures of *R. cordifolia* transgenic for the 35S-*rolB* and 35S-*rolC* genes in comparison to a non-transformed callus culture. The anthraquinone content was shown to be significantly increased in transgenic cultures, thus providing further evidence that the *rolB* and *rolC* genes can act as activators of secondary metabolism in plant cells. Methyl jasmonate and salicylic acid strongly increased anthraquinone accumulation in both transgenic and non-transgenic *R. cordifolia* calluses, whereas ethephon did not. A treatment of the cultures by cantharidin, the protein phosphatase 2A inhibitor, resulted in massive induction of anthraquinone accumulation in the transgenic cultures only. We suggest that the *rolB* and *rolC* genes affect secondary metabolite production in *R. cordifolia* transgenic cells via a cantharidin-sensitive protein phosphorylation mechanism.

P-1158

Development of Edible Vaccine in Carrot Through Engineering the Chloroplast Genome. SHASHI KUMAR and Henry Daniell. Department of Molecular Biology and Microbiology, University of Central Florida, 12722 Research Parkway, Orlando FL 32826–3227. E-mail: daniell@mail.ucf.edu

Carrot (*Daucus carota* L.) is a biennial plant grown for its edible tap root, rich in vitamin A, fiber and is ideal for long-term storage. It will be most suitable for genetic manipulation of colored chromoplasts for the production of edible vaccines. A remarkable feature of chloroplast genetic engineering is the observation of exceptionally large accumulation of foreign proteins (up to 46% tsp) in transgenic plants. Recent achievements made in our laboratory for the hyperexpression of pharmaceutical proteins in tobacco chloroplasts and manipulation of tomato chromoplast genome has potentially motivated us to extend this technology to carrot. For transformation of carrot, flanking sequences (*trnI* and *trnA*) has been amplified with the help of specific PCR primers and duration for *in vitro* regeneration of carrot plantlets has been shortened to four months from eight months after replacing the antibiotic selection with the selectable marker BADH (derived from spinach). Genes like CTB (Cholera Toxin B-subunit) and full length PA (Anthrax Protective Antigen) will be subcloned into the pLD chloroplast transformation vectors in between the homologous recombination sites of the carrot chloroplast genome. Fine cell suspension culture of carrot will be subjected to bombardment of chloroplast vectors. Transgenic carrot plants will be screened on betaine aldehyde. Transgenic carrot should provide a valuable tool for the development of edible vaccines or oral delivery of biopharmaceuticals.

P-1159

Delivery of Subunit Vaccines in Maize Seed. JEFFREY R. LANE[1], Barry J. Lamphear[1], Stephen J. Streatfield[1], Joseph M. Jilka[1], Christopher A. Brooks[1], Donna K. Barker[1], Debra D. Turner[2], Donna E. Delaney[1], Martin Garcia[1], Barry Wiggins[1], Susan L. Woodard[1], Elizabeth E. Hood[1], Ian R. Tizard[2], Bruce Lawhorn[3], and John A. Howard[1]. [1]ProdiGene, 101 Gateway Boulevard, Suite 100, College Station, TX 77845; [2]Department of Veterinary Pathobiology; and [3]Department of Large Animal Medicine, College of Veterinary Medicine, Texas A&M University, College Station, TX 77843. Email: Jlane@prodigene.com

The use of recombinant gene technologies by the vaccine industry has revolutionized the way antigens are generated, and has provided safer, more effective means of protecting animals and humans against bacterial and viral pathogens. Viral and bacterial antigens for recombinant subunit vaccines have been produced in a variety of organisms. Transgenic plants are now recognized as legitimate sources for these proteins, especially in the developing area of oral vaccines, because antigens have been shown to be correctly processed in plants into forms that elicit immune responses when fed to animals or humans. Antigens expressed in maize (Zea mays) are particularly attractive since they can be deposited in the natural storage vessel, the corn seed, and can be conveniently delivered to any organism that consumes grain. We will demonstrate high level expression of the ß-subunit of Escherichia coli heat-labile enterotoxin and the spike protein of swine transmissible gastroenteritis in corn, and have demonstrated that these antigens delivered in the seed elicit protective immune responses. We also provide additional data to support the potency, efficacy, and stability of recombinant subunit vaccines delivered in maize seed.

P-1160

Production of Pharmaceutical Proteins Using a High Expression Promoter in Medicinal Plant Cell Cultures. HAENG-SOON LEE, Sun-Mee Choi, Suk-Yoon Kwon, and Sang-Soo Kwak. Plant Cell Biotechnology Lab., Korea Research Institute of Bioscience & Biotechnology, Oun-Dong 52, Yusiong, Taejon, 305–333, Korea. E-mail: sskwak@mail.kribb.re.kr

Production of pharmaceutical proteins in cultured plant cells has been actively studied due to its potential commercial utility. Only a small number of low molecular components derived from plants such as shikonin and taxol have been successfully produced in plant suspension cultures. However, with recent advance in plant metabolic engineering, protein expression in plant cell suspension cultures has significant potential. A powerful expression system using an appropriate promoter is key requisite for expression of foreign genes efficiently in cultured plant cells. Recently, we have isolated a strong oxidative stress-inducible peroxidase (*SWPA2*) promoter was cloned from suspension cultures of sweet potato (*Ipomoea batatas*). The expression of GUS activity in transgenic tobacco plants under the control of *SWPA2* promoter was strongly induced in response to environmental stresses including hydrogen peroxide, wounding and UV treatment. Furthermore, GUS activity in suspension cultures of transgenic cells derived from transgenic tobacco leaves was strongly expressed following the stationary growth stage. We anticipate the *SWPA2* promoter will be biotechnologically useful for the development of transgenic cell lines engineered to produce key pharmaceutical proteins. In the presentation, the possible production of pharmaceutical protein using *SWPA2* promoter in medicinal plant cell will be introduced.

P-1161

Suppression of Autoimmune Diabetes by the Use of Transgenic Plants Expressing Autoantigen Glutamic Acid Decarboxylase (hGAD65) and Immunoregulatory Cytokines to Induce Oral Immune Tolerance. SHENGWU MA, Y. Huang, and A. M. Jevnikar. Siebens-Drake Research Institute and London Health Sciences Centre and University of Western Ontario, 1400 Western Road, London, Ontario, Canada N6G 2V4. E-mail: shengwu@rri.on.ca

We previously demonstrated that transgenic plants expressing a diabetes-associated autoantigen, mouse glutamic acid decarboxylase (GAD67), when fed to young non-obese diabetic (NOD) mice, a model of human Type 1 diabetes, could prevent diabetes. Protection is due to the induction of oral immune tolerance as characterized by the specific proliferative inhibition of GAD-specific lymphocytes and the induction of cytokine mediated immune regulatory networks and production of Th2 type cytokines. Use of adjuvants can enhance oral immune tolerance, but commonly used mucosal adjuvants such as cholera toxin B subunit (CTB) may be limited by neutralizing immune responses. Immunoregulatory cytokines such as IL-4 may also augment Th2 protective responses. We have developed transgenic plants expressing human GAD65 and murine IL-4. NOD mice given both hGAD65 and IL-4 transgenic plants were protected from diabetes, while those receiving hGAD65 or IL-4 transgenic plants alone were not protected. Protection was associated with the induction of GAD65-specific regulatory cells. These results demonstrate that human GAD65 and IL-4 expressed by transgenic plants can induce protective oral immune tolerance ans is synergistic in preventing diabetes.

P-1162

Broth Rheology and Morphological Properties of *Solanum chrysotrichm* Cultivated in Shake Flasks and a Stirred Tank. M. RODRÍGUEZ-MONROY, G. Trejo-Tapia, J. Trejo-Espino, and A. Jiménez-Aparicio. Departamento de Biotecnología, CEPROBI-IPN. PO. Box 24. Yautepec, Morelos. México 62731. E-mail: mrmonroy@ipn.mx

Cell morphology and aggregates size of plant cell suspension cultures are important because they determine the flow behaviour of broths and, this property is fundamental to define the mixing characteristics of suspension cultures. During the scale-up of cell suspension cultures, a reduction of aggregates size has been reported. The objective of this work was to evaluate the cell morphology of *Solanum chrysotrichum* cultivated in shake flasks (SF) and a 2 L stirred tank (ST) and, to analyze their influence on the flow behaviour of broths. Image analysis was used to know the size and form of aggregates. Elliptical form factor (EFF) was used to characterize the aggregate roundness. The results showed that the cultures of *S. chrysotrichum* developed in ST had an EFF lower than 3, in contrast with the aggregates from SF, which presented a considerable proportion of aggregates with EFF higher than 3. Additionally, the aggregates grown in SF were higher than those present in ST. The broths presented a pseudoplastic behavior determined by the cell concentration. However, a comparative analysis at the same biomass concentration showed that, the broths developed in SF presented higher pseudoplasticity than those of ST. The results of the present work demonstrated that during the scale up of *S. chrysotrichum* cultures, the size and form of aggregates played an important role for the pseudoplastic characteristics.

P-1163

Application of Inducible Plant-Expression Systems for Unstable Proteins. FABRICIO MEDINA-BOLIVAR, Vanessa Funk, and Carole L. Cramer. Fralin Biotechnology Center, Virginia Tech, Blacksburg, VA 24061. E-mail FMB2@VT.EDU

Inducible promoters offer several advantages over constitutive expression systems, allowing for controlled expression of the desirable protein. In this work we are evaluating the use of the MeGA™ (Mechanical Gene Activation, CropTech Corp.) promoter for expression of a model protein, ricin B, that is highly unstable when expressed in the apoplast of tobacco cells. We have previously shown that ricin B, the non-toxic galactose/galactosamine binding subunit of ricin, is an effective mucosal adjuvant/carrier for genetically fused antigens, showing tremendous applications in vaccine development. Spiking experiments with ricin B purified from castor bean into hairy root cultures of tobacco, tomato and *Hyoscyamus muticus* confirmed that the ricin B is highly susceptible to proteolytic degradation in the culture media independent of the plant species. Degradation in all cases occurred after 24 hours of incubation. To optimize expression of secreted ricin B and ricin B fusion proteins in tobacco, we have generated transgenic plants expressing these proteins under the control of the inducible MeGA™ promoter. Initial characterization of the transgenic plants indicated that ricin B can be purified after 24–48 hours of induction and detected by immunoblotting. Hairy roots were established from these plants and are currently under characterization. These results demonstrated the utility of an inducible gene expression system to express highly unstable proteins.

P-1164

Expression of Monoclonal Antibodies in Transgenic Chloroplasts. DEEPIKA MINHAS[1], Amit Dhingra[1], Tanvi Panchal[1], Keith Wycoff[2], and Henry Daniell[1]. [1]Department of Molecular Biology and Microbiology, 12722 Research Parkway, Orlando, FL 32826–3227 and [2]Planet Biotechnology Inc., 25571 Clawiter Road, Hayward, CA 94548. Email: daniell@mail.ucf.edu

Owing to their remarkable specificity and therapeutic nature for defined targets, monoclonal antibodies are emerging as therapeutic drugs at a fast rate. The synthetic monoclonal antibody employed in the present study reacts with certain rotavirus serotypes. Rotaviruses are one of the major causes of scours (diarrhea) in livestock. Scours is not a disease; it is a clinical sign of a disease which may have many causes and death from scours is usually due to loss of electrolytes, changes in body chemistry, dehydration and acid-base balance. The anti-rota antibody is being expressed in transgenic tobacco chloroplasts. The chloroplast genome was chosen for transformation with antibody genes due to tremendously high levels of foreign protein expression; ability to fold, process and assemble foreign proteins with disulfide bridges; simpler purification and transgene containment via maternal inheritance. To enhance translation, a codon optimized gene under the control of specific 5' untranslated regions (UTRs) was used. Transgene integration into the genome has been confirmed by PCR. Further, western analysis will be done to check for multisubunit assembly and ELISA for protein quantification. IgA-G, a humanized, chimeric monoclonal antibody (Guy's 13) has been successfully synthesized and assembled in transgenic tobacco chloroplasts with disulfide bridges. Guy's 13 recognizes the surface antigen Streptococcus mutans, the bacteria that causes dental cavities. In this study, integration into the chloroplast genome was confirmed by PCR and southern blot analyses. Western blot analysis revealed the expression of heavy and light chains individually as well as the fully assembled antibody, thereby suggesting the presence of chaperonins for proper protein folding and enzymes for formation of disulfide bonds within transgenic chloroplasts.

P-1165

Transformation of *Arabidopsis thaliana* with a Protective Antigen for the Tapeworm *Taenia solium*. SIMONE Y. POZNANSKI[1], Carolyn J. Schultz[2], Marshall Lightowlers[3], Richard Strugnell[4], and Tony Bacic[1]. CRC for Bioproducts, PCBRC, School of Botany, University of Melbourne, VIC, 3010 Australia[1]. Dept of Plant Science, Adelaide University, RMB1 Glen Osmond, S, 5024, Australia[2]. Molecular Parasitology Lab, University of Melbourne, Werribee, VIC, 3030 Australia[3]. Department of Microbiology and Immunology, University of Melbourne, VIC, 3010 Australia[4]. E-mail: s.poznanski@pgrad.unimelb.edu.au

Plant based systems for expressing recombinant proteins are gaining popularity because they contain advanced protein folding pathways and glycosylation. The use of plant derived recombinant proteins for therapeutic use has also the advantage of minimizing the potential for viral contamination. Both whole plants and cell suspension cultures are being used and each system has selective advantages. The main advantages provided by whole plant based methods are low maintenance and production cost in comparison to other methods. We wanted to compare the levels of recombinant protein expression using whole plant and cell suspension cultures. The protective antigen (a 14.7 kDa and 22 kDa protein) for *Taenia solium* (a tapeworm that infects humans and pigs), was introduced into the *Arabidopsis thaliana* genome via *Agrobacterium tumefaciens* transformation. Transformed plants were selected using kanamycin resistance. Putative transformants were confirmed by PCR. Positive plants were then analysed for RNA and protein expression. A cell culture was then established from one of the highest protein producing plants.Some modification of the protein in the whole plant is seen including glycosylation since the protein is slightly larger then the expected size. Secreting the recombinant proteins into the media of plant suspension cultures offers the possibility of continual harvesting of recombinant protein and should minimize any deleterious effects the recombinant protein has on the whole plant.

P-1166

High Level Expression of a Fungal Laccase in Transgenic Maize. K. L. RUBY, M. R. Bailey, S. L. Woodard, E. Callaway*, D. Delaney, K. Beifuss, M. Magallanes-Lundback**, J. Lane, M. E. Horn, M. Ward***, F. Van Gastel***, J. A. Howard, and E. E. Hood. ProdiGene, College Station, TX 77845; *Texas A&M University, College Station, TX 77843; **Michigan State University, East Lansing, MI 48824; and ***Genencor Int. Inc., Palo Alto, CA 94304. E-mail:rubyk@prodigene.com

We have expressed active laccase at greater than 40 ppm in recombinant maize seed. Oxidation/reduction enzymes generate highly reactive products because they catalyze electron movement between substrates. Thus, these enzymes have been notoriously hard to express in heterologous systems. By directing expression to the appropriate cellular targets, we were able to increase the amount of active laccase recovered. In addition, we developed methods for reconstituting inactive laccase by including copper in the extraction processes. Laccase produced in maize can be used to modify lignin or other phenolic substrates in a variety of applications including bleaching, detoxification of waste-streams, and polymerization applications.

P-1167

Optimization of Codon Composition and Regulatory Elements for Expression of Human Insulin-like Growth Factor 1 in Transgenic Chloroplasts. GRICEL RUIZ, Henry Daniell, and Puri Fortes. Department of Molecular Biology and Microbiology, University of Central Florida, Orlando, FL 32826–3227. E-mail: daniell@mail.ucf.edu

The human insulin-like growth factor 1 (IGF-1) is a potent multifunctional anabolic hormone produced by the liver. IGF-1 polypeptide is composed of 70 amino acids with a molecular weight of 7.6 kDa and contains three disulfide bonds. IGF-1 is involved in the regulation of cell proliferation and differentiation of a wide variety of cell and tissue types, and plays an important role in tissue renewal and repair. Recent studies have shown that IGF-1 helps in preventing osteoporosis by increasing the absorption of calcium in the bone and also stimulates osteoblast differentiation and proliferation. In addition, it helps increasing the muscle mass and reduce fat in the body. All these and many others application of IGF-1 make it an indispensable therapeutic protein. However, one cirrhotic patient requires 600mg of IGF-1 per year and the cost per mg is $30,000. In the past, IGF-1 has been expressed in E.coli but the protein cannot be produced in the mature form, because E.coli does not form disulfide bonds in the cytoplasm. Transgenic chloroplast technology provides a good solution to recombinant protein production, because of the capability to achieve high expression levels, and the ability to fold and process eukaryotic proteins with disulfide bridges. To increase expression levels, a synthetic IGF-1 gene with optimized codons for tobacco chloroplast was made. Also, different regulatory elements were used to optimeze IGF-1 expression levels. The integration of the IGF-1 gene into the tobacco chloroplast genome was confirmed using PCR and Southern analysis. The IGF-1 protein has been detected in transgenic tobacco chloroplasts by western blot analysis. The protein expression levels will be quantified by ELISA. Results of this investigation will be presented.

P-1168

Application Oriented Research on Pea Seeds in Context to Plants as Bioreactors. I. SAALBACH, H. Bäumlein, U. Conrad, K. Herbers, F. Hosein, J. Kumlehn, and M. Giersberg. Gene transfer group, IPK-Gatersleben, Correnstrasse 3, 06466 Gatersleben, Germany. E-mail: Isolde@ipk-gatersleben.de

Transgenic plants offer an effective and economical alternative for the production of biomolecules and open up new perspectives for agriculture. Especially, the large seeds of cereal and legume crop plants represent promising "bioreactors". If seed specific promoters come into operation, it will be possible to synthesise and accumulate a wide spectrum of biomolecules in pea seeds. Since plants are free of human and animal diseases, the application of plant seeds is especially attractive for the safe and low-cost production of engineered antibodies for medical and veterinary purposes. As a model system for therapeutical biomolecules a scfv-antibody directed against abscic acid (ABA) was expressed exclusively in pea seeds under the control of the promoter of the unknown seed protein (USP) from *Vicia faba*. Pea plants are presented here also as a possible alternative for the production of functional and extremely stable bacterial enzymes under field conditions.The production of technical enzymes (heat stable alpha-amylase from *Bacillus licheniformis* and heat stable xylanase from *Clostridium thermocellum*) in the seeds of commercially grown field pea varieties were also investigated. The expression of a heat stable alpha-amylase of *Bacillus licheniformis*, driven by the seed specific USP-promoter in transgenic homozygous pea was characterised. Results of the field trials in 2000 and 2001 with a homozygous pea line expressing the heat stable alpha-amylase will be summarized. To optimise gene expression in seeds a modified version of the common USP-promoter was designed.

P-1169

Micropropagation: A Tool for the Production of High Quality Plant-based Medicines. PRAVEEN K. SAXENA. Plant Agriculture, Biotechnology Division, University of Guelph, Guelph, Ontario, Canada N1G 2W1. Email: psaxena@uoguelph.ca

Plants are widely used for healing the ailments of body, spirit, and the mind but the "plant" is often the most neglected part of plant-based medicine. The problems with plant-based medicine with serious health consequences include contamination of preparations with microorganisms, toxins, misidentified species, and a lack of understanding of the unique physiology of medicinal plant species. Plant tissue culture techniques offer an integrated approach for the production of standardized quality phytopharmaceutical through mass-production of consistent plant material for physiological characterization and analysis of active ingredients. Micropropogation systems have been successfully developed for several medicinal species such as St. John's wort, (*Hypericum perforatum*), Echinacea sp., Huang-qin (*Scutelleria baicalnesis*), *Echinops spinossimus* and *Artemisia judaica*. Regeneration occurred via organogenesis and embryogenesis in response to thidiazuron, NAA and BAP. Biochemical characterization of St. John's wort revealed the presence of a pathway for synthesis of the mammalian neurohormones serotonin and melatonin. Studies with inhibitors of this pathway provided preliminary evidence that alterations in the relative ratio of melatonin to serotonin can alter plant developmental responses, especially those responses associated with auxin and light. In further research, in vitro protocols were adapted for the large-scale optimized production of individual metabolites in various types of bioreactors. In St. John's wort cultures, the concentration of indoleamines and the commercially important compounds hypericin, hyperforin and pseudohypericin varied with the culture conditions, type of tissue, and stage of plant development. The integrated approaches of our culture systems will provide the basis for the future development of novel, safe, effective, and high-quality products for consumers.

P-1170

Developing the Plastid as a Potential Vaccine Production System. KAREN A. SHIEL, P. J. Dix, and J. Nugent. Plant Molecular Biology Laboratory and Plant Cell Culture Unit, NUI Maynooth, Co. Kildare, Ireland. E-mail: KAREN.A.SHIEL@may.ie

Oral delivery of vaccines is a proven route for the generation of protective immunity against certain pathogens. A relatively simple and cost-effective method of producing large amounts of protein antigens for vaccination is to produce them in plants. Transgenic plants offer many advantages as vaccine delivery systems. It has been previously demonstrated that antigens could be delivered in nuclear transformed plants, illiciting immune responses in mice and humans. However, transformation of the plastid genome has several advantages over nuclear transformation. Maternal inheritance of the plastid in most plant species prevents pollen-mediated out-crossing. Poloycistronic operon expression occurs naturally in the plastome, thus plastid transformation makes possible the stacking of genes in one transforming unit. There are usually no position effects as the genes of interest are introduced into the plastome via homologous recombination. Finally, the high ploidy level of the plastid genome, up to 10,000 plastid genome copies per leaf cell, allows for increased levels of transgene expression. The aim of this project is to introduce the haemagglutinin (HA) gene of influenza virus into the chloroplasts of *Nicotiana tabacum*. Haemagglutinin is the major surface antigen of the influenza virus. Two methods of plastid transformation are being utilized in this study: Polyethelyne-glycol (PEG) mediated transformation of tobacco protoplasts and Biolistic particle bombardment of intact leaves. Several PEG-mediated transformations were performed resulting in spectinomycin resistant calli. Several Biolistic transformations have also been carried out and the results of these transformations will be presented at the conference. Ultimately, we hope to assess immunogenicity of the plant-based antigen in a murine model.

P-1171

Development of Transgenic Plants as Source of Edible Vaccine for Peste Des Petis Ruminants, an Animal Disease. K. J. M. VALLY, K. Abha, M. S. Shaila, and G. Lakshmi Sita. Department of Microbiology and Cell Biology, Indian Institute of Science, Bangalore-560 012, India. E-mail: vallykmv@rediffmail.com

Peste des petits ruminants is a highly contagious disease of sheep, goats and wild ruminants, with a high mortality rate. The causative agent, *peste des petits ruminant* virus (PPRV) has only one antigenic type (serotype) an attenuated, live vaccine with high immunogenicity is available. It belongs to the family paramyxoviridae and genus mobillivirus. PPRV hemagglutinin protein 'hemagglutinin nuraminidase' (HN) affects small ruminants by absorbing them through unknown biological activity. PPRV is highly immunogenic and surviving animals are immune to the disease for the rest of their lifetime. *Peste des petits ruminants* disease has been eradicated in developed countries, but is still prevalent in parts of Africa, the middle East and South Asia, where eradication campaigns are underway. The major drawback of the currently used vaccine is its heat lability. Recombinant vaccine based on cell culture based techniques is prohibitively expensive for large-scale vaccination programs. Transgenic plants that express protective antigens of PPRV in their edible tissues might be used as an inexpensive oral vaccine production and delivery system. Towards this goal, we have undertaken to develop (a) transgenic tobacco (model system) and (b) transgenic peanut (*Arachis hypogea*) plants expressing the hemagglutinin nuraminidase (HN) protein of PPR virus. We present here our results in developing transgenic plants of tobacco and peanut expressing the HN protein. The hemagglutinin nuraminidase gene from pGemT was sub-cloned into the binary vector pBI 121. The recombinant binary vector pBI-HN was mobilized into the hypervirulent *Agrobacterium tumefaciens* strain GV3101. For tobacco, leaf disc and for peanut embryonal axies was used as the initial explant for regenaration. Presence of HN gene in was shown by PCR using gene specific primers. The integration of the transgene has been confirmed by Southern hybridization. The expression of HN protein in transgenic lines has been shown using polyclonal monospecific antibody to HN. HN protein in plant extracts was quantified by DAS – ELISA.

P-1172

Induction of Hairy Roots In Vitro in (*Physalis peruviana* l.) to Obtain Secondary Metabolites. M. VELASQUEZ LOZANO and M. Perea Dallos. Departamento de Ingeniería Química, Universidad Nacional de Colombia, P.O. Box 14490, Bogotá – Colombia. Email: mvelasq@ing.unal.edu.co, mapere@ibun.unal.edu.co

Uchuva is a plant originated from the Andean. Its fruit is consumed mainly as a fresh product and for the case of Colombia, it has reach high levels of export. Nutritionally, the fruit is considered a good source of vitamin C and beta-carotene. The effect of exogenously fed hormones on hairy root cultures of *Physalis peruviana* L was studied. Preliminary essays were done by using young leaves, from plants grown under roof, as explants. M&S (1962) medium with agar Merck (r), 30 g/l sucrose and pH 5.8 was employed to initiate rooting. Different type of auxins were tested among others: indole-3-acetic acid IAA,naphtolene acetic acid NAA, 2,4-dichlorophenoxyacetic acid 2–4 D and 3,6 dichloro o anisic acid dicamba. IAA and NAA were chosen to be studied in liquid media. Incidence of auxin concentration, agitation and oxygen concentration on seedling and hairy root growing was analyzed. Presence of secondary metabolites was also determined by analytical methods as HPLC.

P-1173

Brazzein, a Recombinant Protein with a Sweet Future. A. VINAS, Z. Nikolov, P. Irwin, B. Lamphear, D. Delaney, S. Streatfield, J. Jilka, K. Beifuss, J. A. Howard, and E. E. Hood. ProdiGene, 101 Gateway Blvd., Suite 100, College Station, TX 77845. E-mail:mherbert@prodigene.com

Brazzein is a small sweet protein from an African berry, the native Pentadiplandra brazzeana fruit. The protein is 54 amino acids in length with a molecular weight of approximately 6,000 Da. Three forms of brazzein have been identified that differ in their N-terminal amino acid residue: 1) pyro-glutamate, 2) glutamine, and 3) truncated by 1 amino acid (glutamine/glutamate). Type 1 forms spontaneously from type 2 and is less sweet. Using constitutive and seed-preferred promoters, we have expressed the gene for type 2 brazzein in transgenic maize. The best expression (greater than 2% of total soluble protein) was seen in seed in which a seed-preferred promoter was used and the protein directed to the cell wall. In addition, the integrity of the protein is maintained as demonstrated by western blot. We are developing production lines through our back-cross program to improve agronomic quality for production. We have harvested a crop of grain in summer 2001 for protein purification for taste tests. Brazzein produced in Pichia fermentation cultures delivers a full, sugar-like mouth feel, and is approximately 500–1000X as sweet as sugar. Its applications are in making low calorie baked and snack foods. This work was supported in part by USDA SBIR grant #00–33610-9435

P-1174

Expression of *Bacillus anthracis* Protective Antigen in Transgenic Chloroplasts Towards the Development of an Improved Vaccine or an Edible Vaccine. J. WATSON, A. Dhingra, and H. Daniell. Department of Molecular Biology and Microbiology, University of Central Florida, Orlando, FL 32829-3227. Email: daniell@mail.ucf.edu

Bacillus anthracis is the causative agent for the disease anthrax which has become a real threat today due to bioterrorism. Large-scale production of immunogenic Protective Antigen (PA encoded by the gene *pag*) is necessary to have sufficient vaccines available in times of crisis. By expressing *pag* in plants, the toxin contaminants associated with the current vaccine (which is made from a filtrate of actual *B. anthracis*) would be eliminated resulting in a cleaner vaccine. Using chloroplast transformation technology, large quantities of PA can be produced in transgenic plants because of the ability to express 10,000 copies of the transgene per cell. *Pag* was cloned into the universal chloroplast vector along with *psbA* 5'UTR to enhance *pag* expression. Previous work in our lab with another *Bacillus* protein utilized ORF1,2 from *cry2Aa2* operon to achieve higher expression (46% tsp). Therefore, a second construct was made with ORF1,2 and *psbA* 5' UTR to determine if even greater expression might be obtained. Chloroplast integration of the transgene has been confirmed by PCR. Further characterization of the transgenic lines will be done including protein quantification by ELISA. A novel approach for an improved anthrax vaccine is an edible vaccine. We plan to achieve high level expression in chromoplasts of tomato fruits through chloroplast transformation and use of a selectable marker gene from spinach (BADH) instead of antibiotic resistant genes. Integration regions in tobacco and tomato are similar. Results of these investigations will be presented.

P-1175

Expression of Human Epidermal Growth Factor (hEGF) by Integrative and Non-Integrative Systems in Tobacco Plants. SONIA WIRTH[1], Gabriela Calamante[3], Alejandro Mentaberry[1], Leonardo Bussmann[2], Lino Barañao[2], and Fernando Bravo Almonacid[1]. [1]Instituto de Investigaciones en Ingeniería Genética y Biología Molecular, INGEBI-UBA-CONICET. Obligado 2490 piso 2, Buenos Aires, Argentina; [2]Instituto de Biología y Medicina Experimental, IByME-CONICET Obligado 2490, Buenos Aires, Argentina; and [3] Instituto Nacional de Tecnología Agropecuarria. INTA Castelar, Argentina. E-mail: fbravo@dna.uba.ar

Features as the low cost of scaling-up, the eukaryotic post-translational modifications and the reduced risk of mammalian pathogen contamination have promoted the study of transgenic plant systems as biorreactors for the production of recombinant proteins. In this work we used integrative and non-integrative expression systems to the production of a human therapeutic protein, the human Epidermal Growth Factor (hEGF), in tobacco plants. This factor has been related to cellular differentiation, and tissue protection and repair. In preliminary studies, tobacco plants were transformed with the hEGF gene under the CaMV 35S promoter, but accumulations levels do not exceed 0.0001% of total soluble proteins. With the aim to improve the yields of hEGF, two strategies were followed for its expression. First we use a modified CaMV 35S promoter in which transcription enhancer was duplicated to increase promoter strength, and also contain the translational enhancer sequence omega from TMV. Second, the signal sequence from AP24 (a tobacco osmotin) was fused in frame to the hEGF sequence with the purpose of exporting it to the extracelular space. As in the cytoplasmic versions, the apoplastic constructions were cloned under the wild type CaMV 35S promoter sequence or the modified promoter plus the omega sequence. Genetic constructions in which 17 residues from AP24 remains fused to hEGF and a version in which the hEGF initial metionine is replaced by an alanine residue corresponding to the +1 position from the endoplasmatic peptidase processing site were made. All the constructions were cloned in the binary vector pBI121 and introduced into *Nicotiana tabacum* var. *Xanthi D8* plants by *Agrobacterium tumefaciens* mediated leaf disc transformation. The transgenic nature of each plant was verified through PCR and Southern-blot assays and the expression of recombinant hEGF was measured by an ELISA assay using commercial antibodies. The yields in plants transformed with the cytoplasmatic versions were comparable and no significative differences were found between the constructions with the CaMV 35S wild type promoter and the modified version. However, the plants transformed with the apoplastic constructions showed a considerable increase in yields which in some cases reached 0.11% of total soluble proteins. The extracelular fluid from plants with the highest expression was used to test the biological activity of the recombinant hEGF. Cell proliferation assays revealed that plant-produced hEGF responds as commercial rhEGF, stimulating NIH 3T3 cell division. By other hand, both the hEGF sequence and the AP24-hEGF fusion were cloned under the duplicated coat protein promoter in a PVX based viral vector developed by Dr. Calamante. RNA transcripts of these vectors and of PVX wild type were used to infect *Nicotiana benthamiana* plants and systemic production of hEGF quantified by an ELISA assay as in the transgenic plants. In the cytoplasmic version of the viral vector hEGF levels were hardly detected, but in the apoplastic viral vector yields reached 0.015% of total soluble proteins. *Vector containing modified CaMV 35S promoter; sequence W from TMV and AP24 gene was a kindly gift from Dr Lázaro Hernández CIGB, Cuba. This work was supported by the Agencia Nacional para la Promoción de la Ciencia y la Tecnología BID 802/OC-AR PICT 98 nº 08–03529.*

P-1176

Toward the Metabolic Engineering of Post-Biosynthetic Events in Plant Cell Cultures. W. ZHANG*[1,2] and C. Franco[1]. [1]Cooperative Research Center for Bioproducts, Flinders University, SA 5042, Adelaide, Australia; [2]Marine Bioproducts Engineering Group, Dalian Institute of Chemical Physics, Chinese Academy of Sciences, Dalian, 116023, China. E-mail: Wei.Zhang@flinders.edu.au

Plant cell cultures have been suggested as a feasible technology for the production of a myriad of plant-derived metabolites. Commercial application of plant cell culture however has met limited success with only a handful of metabolites produced in pilot- and commercial-scales. To improve the production of secondary metabolites in plant cell cultures, many efforts have been devoted to the optimization of biosynthetic pathway by means of both the process engineering approach and the genetic engineering approach. Given that secondary metabolism is the synthesis, metabolism and catabolism of endogenous compounds by the specialized proteins, this review intends to draw attentions on the manipulation and optimization of post-biosynthetic events that follow the formation of core metabolite structures in biosynthetic pathway. These post-biosynthetic events that include post-biosynthetic modifications (chemical/enzymatic), transport, storage/secretion and catabolism/degradation have been largely unexplored in the past. If one looks at the secondary metabolism as a whole, these post-biosynthetic steps will most likely become rate-limiting when the production of secondary metabolites in plant cell cultures is increased to a level that is not normally achieved in the growing plants. This indeed provides great opportunities for the advancements in plant cell culture technology. Without a holistic optimization and manipulation of the post-biosynthetic events, an optimum production process can not be achieved due to the interactions between the biosynthetic and post-biosynthetic steps. In this review, potential areas are discussed on the manipulation of post-biosynthetic events in secondary metabolism where further research is needed to answer fundamental questions that have implications on advanced bioprocess design. Anthocyanin production by plant cell cultures is used as a case study for the discussion. It is perceived that this may represent future opportunities for further advance in commercial plant cell cultures.

P-1177

Effects of Major Nutrients, Exogenous Hormones, Elicitors for Enhancement of Triterpene Glycosides Production in *Centella asiatica* L. Urban Whole Plant Cultures. J. C. AHN*, O. T. KIM, K. S. KIM, and B. HWANG. Department of Biological Sciences, Chonnam National University, Kwangju, Korea 590–711; *Department of Life Sciences, Seonam University, Namwon, Korea 500–757. E-mail: jcahn@tiger.seonam.ac.kr

Centella asiatica (L.) Urban is a plant belonging to the Umbelliferae family, hypocotyl order, which grows in sub-tropical regions as in parts of india, Sri Lanka, China, Indonesia, Malaysia, Australia and Southern and Central Africa. It has been used in traditional medicine in India for treatment of leprosy, varicose veins, ulcers, lupus and certain eczemas, and of mental retardation since prehistoric times. It has recently become popular in all the world because of its significant mental and physical revitalization properties and is marked by Syntex EU and Canada under the trade name Madecassol for the treatment of various dermatological lesion including burns. It is presently being progressed in quite extensive experimental and clinical investigations for other physiological actions such as the sedative, analgesic, antidepressive, antimicrobial, antiviral and immunomodulatory effects. In Korea, most of the phamaceutical companies depend on import from foreign countries because this plants are grown only at the limited coastal area of southern islands. Based on these reason, we had tried to produce the useful compounds, triterpene glycosides from tissue culture(cell suspension, adventitious root and transformed hairy root culture). However, cell or root culture were not able to produce triterpene glycosides, and it seems likely that the biosynthesis of these compounds are related to aerial parts, leaf or stem. So, we had examined the possibility of the whole plant culture in a mass scale and ascertained it. In addition, using whole plants culture, we investigated the optimal concentration of major nutrients and effects of exogenous hormone and some elicitors. Considering the productivity of triterpene glycosides, it is concluded that concentrations of 25 mM KNO_3, 1 mM NaH_2PO_4, 10 mM $MgSO_4$, 0.2 mM $CaCl_2$ in B5 liquid basal media, addition of 0.1 mg/L Thidiazurone(TDZ) among exogenous hormone tested and the treatment of 0.1 mM methyl jasmonate and 0.1 g/L yeast extract showed the most effective and/or enhancement results. As the results, *Centella asiatica* whole plant culture was affirmitive in possibility for obtaining of triterpene glycosides and also on experimetal material for study of biosynthetic pathway.

P-1178

Root Specific Elicitation and Antimicrobial Activity of Rosmarinic Acid in Hairy Root Cultures of Sweet Basil (*Ocimum basilicum* L.). HARSH PAL BAIS[1], Travis S. Walker[1], Herbert P. Schweizer[2], and Jorge M. Vivanco[1]*. [1]Department of Horticulture and Landscape Architecture [2]Department of Microbiology, Colorado State University, Fort Collins, CO 80526. *Corresponding Author. Email: jvivanco@lamar.colostate.edu

Rosmarinic acid (RA) is a multifunctional caffeic acid ester present in sweet basil (*Ocimum basilicum* L.). Roots of *O. basilicum* harbored the maximum titers of RA compared to leaves and shoots. Hairy root cultures of *O. basilicum* transformed with *Agrobacterium rhizogenes* (ATCC-15834) produced constituent levels of RA. Transformed roots showed better growth and RA production compared to the untransformed normal roots. Upon elicitation with fungal cell wall elicitors (CWE) from *Phytophthora cinnamoni*, the production of RA was enhanced. When the biological activity of this compound was tested against soil-borne bacteria and fungi a wide range of inhibitory effects were recorded. Roots were induced to exude RA by fungal challenge, to our knowledge an undocumented observation. Absence of RA in the root exudates of unchallenged root cultures proves that RA under normal circumstances is not exuded. RA showed a wide range of antimicrobial activity against soil-borne microorganisms, with its most deleterious effects against *Pseudomonas aeruginosa*, an opportunistic soil bacterium and human pathogen, suggesting a gap bridged between human pathogen attack on plants or *vice versa*. Confocal and scanning imaging of *Aspergillus niger* hyphae treated with RA (250 μM) exhibited damaged cytoskeletons with broken interseptas and convoluted cell surfaces resulting in a multinucleated stage compared to the untreated control. Both strains of *Pseudomonas aeruginosa* tested, PAO1 and PA14, showed increased spatial division and condensation of DNA upon RA treatment as compared to the untreated control. Biologically, the findings suggest that in nature RA is a constitutive antimicrobial compound released into the surrounding rhizosphere upon microbe challenge.

P-1179

Expanding the Phytoremediation Repertoire of Mercuric Ion Reductase. REBECCA SWANSON BALISH and Richard B. Meagher. Department of Genetics, The University of Georgia, Athens, GA 30602. E-mail: rbalish@arches.uga.edu

Toxic organic and heavy metal pollution of soil and water are serious ecological problems facing populations today, particularly in developing nations. Current means of heavy metal decontamination rely on the removal and disposal of contaminated soil to distant locales, an expensive and ecologically damaging venture. Alternatively, phytoremediation involves the use of plants to extract, detoxify, and/or sequester elemental environmental pollutants from soil and water. Plants possess many characteristics that make their use an attractive alternative to current methods of remediation. The extensive root systems of plants ensure that tons of soil can be detoxified, and photosystem I gives plants excess reducing power not available in other organisms. We have taken a genetic approach to the problem of heavy metal contamination by genetically modifying plants to express bacterial genes involved in the detoxification of ionic mercury (Hg(II)). When the gene for mercuric ion reductase (*merA*) is expressed in a number of diverse plant species, we have found the transgenic plants to be much more resistant to Hg(II) than wild type plants. MerA catalyzes the NADPH-dependent reduction of ionic mercury to the less toxic metallic mercury. Previous work has demonstrated that the MerA protein reduces other metal ions (Ag(I) and Au(III)), albeit at lower efficiencies. We have used directed evolution to alter the *merA* gene in order to generate proteins that reduce a number of toxic heavy metal ions. The mutated *merA* genes were expressed in a metal-sensitive yeast strain in order to screen for novel substrates for the MerA enzyme. Candidate mutant *merA* genes which reduce novel metal ions will subsequently be expressed in plants for use in metal ion detoxification and hyperaccummulation.

P-1180

Phytic Acid in Azolla and Its Role in Metal Binding. RONY OREN BENAROYA and Elisha Tel-Or. The Hebrew University of Jerusalem, Faculty of Agricultural, The Institute of Plant Sciences, P.O. Box 12, Rehovot 76100, Israel. E-mail: Benaroya@agri.huji.ac.il

Our laboratory has been focused on the characterization and application of active uptake and passive absorption of metals by the aquatic fern *Azolla filiculoides*. The content of cadmium, copper, nickel, zinc, chromium, iron and uranium in *Azolla* plants grown in the presence of 10 mg/l metal attained *Azolla* dry weight up to 5–6 mg metal/g. In the present study we suggest that phytic acid- phytate is involved in the accumulation of metals. Phytic acid is the form of phosphate storage in seeds of higher plants, and represents 60 to 90% of their total seed phosphate content. Phytate is also found in vegetative plant tissues. The content of phytate in *Azolla* is 0.3% of *Azolla* dry weight (10% of total phosphate content in *Azolla*). Addition of 20 mg/ml zinc or 0.1M NaCl to the growth medium increased the phytate content in *Azolla* by 20% and 50%, respectively. We extracted phytate from *Azolla* and demonstrated *in vitro* high binding affinity to the heavy metals iron, copper and zinc and low affinity to cadmium. Accumulation of L-*myo* inositol 1-phosphate synthase (INPS), a key enzyme involved in the biosynthesis of phytate and phytase enzyme involved in the brake down of phytate, has been studied. When *Azolla* plants were grown 4 days in addition of 70, 140 and 280 mg/ml NaH_2PO_4 to the growth medium, INPS content in the *Azolla* plants increased by 100%, 400% and 900%, respectively. Addition of 140 and 280 mg/ml NaH_2PO_4 reduced phytase content to 40% and 16% of the control, respectively. Addition of 20 mg/ml zinc, 20 mg/ml cadmium and 0.1M NaCl to the growth medium for 4 days increased INPS content in the *Azolla* plants by 100%, 350% and 500%, respectively. Cadmium and NaCl treatment reduced phytase content to 25% and 10% of the control, respectively.

P-1181

Transgenic *Chlorella* as a Phytoremedial Bioreactor. P. R. COHILL and A. C. Cannons. Department of Biology, University of South Florida, Tampa, FL 33620. E-mail:pcohill@chuma.cas.usf.edu

Chlorella, a unicellular green alga, can serve to remediate polluted aquatic environments. Water from these environments can pass through a packed-bed bioreactor in which *Chlorella* cells grow and be detoxified as the plant cells phytoremediate. The ability of these cells to phytoremediate can be enhanced by the addition of exogenous genes to the cells' genomes. When expressed, these transgenes allow *Chlorella* cells to have increased phytoremediant abilities. Through microprojectile bombardment, *Chlorella* incorporates exogenous genetic material and stably expresses it. Exogenous or native genes normally expressed in the nucleus can be isolated and inserted into a transformation vector designed specifically for homologous recombination with the chloroplast genome. This vector contains spacer sequences found in a transfer RNA gene cluster of the chloroplast genome; these sequences surround a gene for streptomycin resistance as well as a multiple cloning site. Thus, resistance to this antibiotic indicates stable incorporation of the transgenes into the chloroplast genome. Additionally, many copies of the chloroplast genome exist in each cell, meaning that overexpression of the transgenes from multiple genomic copies is possible. One potential transgene expresses phytochelatin synthase (PCS), an enzyme found in plants which produces short peptides, phytochelatins, in response to heavy metal exposure. These phytochelatins bind heavy metal ions and sequester them within the cell. Phytochelatins produced by *Chlorella* cells in response to heavy metal exposure are identifiable through HPLC analysis, indicating the presence of an active PCS gene in the nuclear genome. The native *Chlorella* PCS gene can be identified through expression cloning of a cDNA library in yeast, and can be compared to the recently isolated wheat and *Arabidopsis* forms of the gene through sequence analysis. A chloroplast transformation vector containing a PCS gene can then be used to insert the gene into the *Chlorella* chloroplast genome, resulting in the overexpression of PCS and optimizing the algal cells as bioreactors that serve to phytoremediate.

P-1182

Production of the *Bixa orellana* L. (Achiote) Pigment from Cell Suspension Culture. S. CUARTAS CHACÓN[1], M. Perea Dallos[2], and D. Montoya Castaño[1]. [1]Instituto de Biotecnología, Universidad Nacional de Colombia, P.O. Box 14490, Bogotá – Colombia and [2]Departamento de Biología, Universidad Nacional de Colombia, Bogotá – Colombia. E- mail: soniabix@yahoo.com, mapere@ibun.unal.edu.co, domonto@ibun.unal.edu.co

The *Bixa orellana L.* (achiote, annatto), is a tree from which is extracted the bixin, a natural pigment largely used in foods and cosmetics. The pigment extracted from the achiote is one of the pigments approved by the Worl Healt Organization. There is a very few reports studies of "in vitro" culture of this plant and this is the first study on the pigment production in cells suspension culture reported in Colombia. In this research we used two month seedling growed in a nursey at 27 ± 1 °C, 7 cm of height, plantlets were disinfected with Isodine (r) 3% v/v and sodium hypochlorite 1% v/v. Callus was obtained using a factorial experiment 4x2 (four growing regulators at high and low levels 1 and 3 mg/L and three sources of explants: roots, leaves and hypocotyls). Callus from segments of hypocotyls growing in M & S medium (1962) supplemented with 30grs/L sucrose, agar-agar Merck (r) 7g/L, 2,4-dichlorophenoxyacetic acid; (2,4 – D, 1 mg/L), kinetine (1 mg/L) and adenine (100 mg/L), at 5.8 pH and 26 ± 1 °C in the dark were selected to continue the study. In order to obtain a major cell growing from callus the M & S medium was optimized by using a fractionated factorial design 2^{7-3}. The callus have been divided in 128 parts and currently it is possible to see a light yellow pigment production increased through a somaclonal variation. We found in two of the samples a clear red pigment. This project is in progress.

P-1183

Resistance and Accumulation of Arsenic by Plants Expressing Bacterial Arsenate Reductase and Gamma-glutamylcysteine Synthatase. OM P. DHANKHER[1], Y. Li[1], B. P. Rosen[2], D. Salt[3], and R. B. Meagher[1]. [1]Department of Genetics, University of Georgia, Athens, GA 30602; [2]Department of Biochemistry and Molecular Biology, Wayne State University, Detroit, MI; and [3]Department of Biological Sciences, University of Purdue, West Lafeyette, IN.

Arsenic pollution affects the health of millions of people, but few ecologically sound remedies to arsenic contamination of soil and water have been proposed. We have developed a genetics-based phytoremediation strategy based on controlling the electrochemical state of arsenic in leaves and thiol-sinks for arsenite, making use of two bacterial genes. The model plant *Arabidopsis* was transformed with the *E. coli* arsenate reductase (*ArsC*) gene controlled from a strong light induced rubisco promoter (*SRS1p*). Strong expression of ArsC protein was observed in leaves, but not roots. *SRS1p/ArsC* expressing plants reduced arsenate to arsenite more efficiently than control plants, and were consequently arsenate hypersensitive relative to wild-type. The *E. coli* gene for gamma-glutamylcysteine synthatase (*gamma-ECS*) was expressed in *Arabidopsis* from a strong constitutive actin promoter (*ACT2p*). *A. thaliana* plants expressing *ACT2p/ECS* were highly tolerant to arsenic relative to wild-type. Plants expressing both *SRS1p/ArsC* and *ACT2p/ECS* show significantly greater arsenic resistance than either single transformed plant or wild-type. They accumulated 4 and 17 times more fresh shoot weight as compared to gamma-ECS and ArsC plants, respectively. These hybrid plants expressing *SRS1p/ArsC* and *ACT2p/ECS* accumulated more arsenic in the aboveground tissues as compared to *SRS1p/ArsC*, *ACT2p/ECS* and wild-type plants.These results suggest that it will be feasible to engineer arsenic resistance and accumulation in conservation species for commercial phytoremediation

P-1184

Hyoscyamine or Calystegines? Regulation of Tropane Alkaloid Metabolism. BIRGIT M. DRÄGER. Martin-Luther University Halle-Wittenberg, Institute of Pharmaceutical Biology, Hoher Weg 8, D-06120 Halle, Germany. E-mail: draeger@pharmazie.uni-halle.de

The tropane alkaloid pathway produces the medicinally important alkaloids hyoscyamine (racemic mixture: atropine) and scopolamine. Calystegines are polyhydroxyl nortropane alkaloids that also derive from this pathway. Calystegines occur in Solanaceae, that are known for their tropane alkaloid content, Atropa belladonna, Hyoscyamus niger, Datura stramonium, and they accumulate in some Convolvulaceae, their name comes from Calystegia sepium. Calystegine occurrence varies, both, qualitatively and quantitatively in different plants and plant organs. Solanaceae that were thought not to contain tropane alkaloids, i.e. potato and tomato, also accumulate calystegines. Calystegines show glycosidase inhibitory activity and have a high therapeutic potential. Putrescine methyl transferase PMT is the first specific enzyme of the tropane alkaloid biosynthesis. The enzyme protein and the gene resemble those of spermidine synthase from which PMT may have originated during tropane alkaloid evolution. The reduction of tropinone is a branch point in the biosynthesis. The two reduction products, tropine and pseudotropine, are isomeric alcohols that are formed by specific tropinone reductases. Tropine is incorporated into hyoscyamine and pseudotropine is the metabolite leading to calystegines. ^{15}N-labelled tropinone was traced to flow through the pathway via pseudotropine into calystegines. Partition of the substrate tropinone between hyoscyamine and calystegines is assumed to be regulated by the activities and localization of the two tropinone reductases. The metabolite flux through the tropane alkaloid pathway appears strictly regulated, enzyme expression and product accumulation appear in a time and organ specific pattern. Both tropinone reductases were isolated and the genes were cloned from several Solanaceae. They show catalytic similarities and sequence homology and are believed to have derived from a common ancestral short chain reductase. Further putative points of regulation of metabolic flux are the demethylation and hydroxylation of the nortropane ring leading to calystegines and the formation and esterification of tropic acid starting from phenylalanine and ending in hyoscyamine. In summary, the tropane alkaloid pathway appears to be recruited from genes and enzymes of primary metabolism and regulated at several bottlenecks by enzyme expression and activity and substrate availability.

P-1184A

Synthesis and Accumulation of Antioxidant Phenolic Compounds in *In Vitro* Cultures of Sage (*Salvia officinalis* L.) (*). Paula C. Santos-Gomes[a], Rosa M. Seabra[b], Paula B. Andrade[b], MANUEL FERNANDES-FERREIRA[a]. (a)Department of Biology, University of Minho, Campus de Gualtar, 4710-057 Braga, Portugal and (b)CEQUP/Pharmacognosy Laboratory, Faculty of Pharmacy, University of Porto, R. Anibal Cunha, 4050 Porto, Portugal. E-mail: mfferreira@bio.uminho.pt

In order to study the synthesis and accumulation of phenolic antioxidant compounds in *in vitro* cultures of sage, shoots, *calli* and suspended cells of this species were established under different auxin and citokinin supplementations by culturing nodal segments excised from aseptic seedlings germinated *in vitro*. Stabilized *calli* and suspended cells were established in the presence of dichlorophenoxyacetic acid (2,4-D) with benzyladenine (BA) and 2,4-D with kinetin (KIN) respectively. Shoot proliferation, at high rates, and good shoot growth occurred in the presence of BA and 2,4-D. However, in these conditions, the accumulation of total antioxidant phenolics was the lowest. Through HPLC-DAD, seventeen compounds were identified in the antioxidant phenolic extracts from shoots: gallic acid, 3-O-caffeoylquinic acid, 5-O-caffeioylquinic acid, caffeic acid, and rosmarinic acid, as phenolic acids; hesperetin, apigenin, hispidulin, cirsimaritin, and genkwanin, as flavonoids; epirosmanol, epirosmanol methylethyl ether, carnosol, epiisorosmanol ethyl ether, rosmadial, carnosic acid, and methyl carnosate, as phenolic diterpenes. From these seventeen compounds, *calli* and suspended cells accumulated, in measurable amounts, only fourteen and seven respectively. Rosmarinic acid and carnosol were the main compounds in the antioxidant phenolic extracts from shoots. (*) Project supported by POCTI/AGR/43482/2001.

P-1184B

Variation of the Essential Oil Profile of Peppermint (*Mentha piperita* L.) In Vitro Shoot Cultures (*). ANA P. GUEDES and Manuel Fernandes-Ferreira. Department of Biology, University of Minho, Campus de Gualtar, 4710-057 Braga, Portugal. E-mail: mfferreira@bio.uminho.pt

In order to follow the profile of the essential oil composition during a culture cycle of peppermint, samples were taken at the end of the 2nd and 5th days of the subculture and thereafter with intervals of 5 days during a total period of four months. Shoot samples were hydrodistilled and the respective essential oils were analysed by gas chromatography (GC) and gas chromatography mass spectrometry (GC-MS). The maximum number of compounds (19-20) were found in samples obtained after the 50th day. All the compounds were terpenes, three of them sesquiterpenes and the others monoterpenes including monoterpene hydrocarbons and oxygen-containing monoterpenes. During the first 25 days the number of terpenic compounds in the samples varied from 10 to 14. From the compounds present, in measurable amounts, in all samples, the major ones were menthofuran (51-78%), pulegone (12-32%), menthyl acetate (0.3-7.3%), limonene (1.2-6.1%), isomenthone (0.2-3.6%). L-Menthol and its direct precursor, L-menthone, which are the first and the second major compounds, respectively, in the essential oils of the peppermint in vivo plants, representing together more than 50% of it, in the respective in vitro shoots were among the least represented ones. As menthofuran is the only compound whose variation was clearly correlated with that of pulegone, a good explanation for the too low levels of L-menthol and L-menthone in in vitro peppermint shoots, seems to be the deficient expression or activity of the redutase enzymes responsible for conversion of pulegone to L-mentone and this in L-menthol. (*) Project supported by POCTI/AGR/43482/2001.

P-1184C

Epicuticular Wax from In Vitro Shoots of *Euphorbia characias* L. Maria C. F. B. Viana-Ferreira[1] and M. FERNANDES-FERREIRA[2]. [1]Esc. Sec. Carlos Amarante, 4700, Braga, Portugal; [2]Department of Biology, University of Minho, Campus de Gualtar, 4710 - 057 Braga, Portugal. E-mail: mfferreira@bio.uminho.pt

Epicuticular wax constitutes a waterproofing interface between plant tissue and the growth environment. Aseptically *in vitro* grown shoots of *Euphorbia characias* L. were obtained by regeneration from wild plant axillary buds cultivated on MS medium. Shoots were maintained through subcultures of shoot segments performed at intervals of 10 weeks on the same MS basal medium supplemented with IBA and KIN. A sample of 10 culture flasks was taken at the end of the second subculture for extraction and analysis of the shoot epicuticular waxes. The fresh weight of the whole shoot biomass was determined before epicuticular extraction and the dry weight was determined after wax extraction and freeze drying of the biomass. The GC and the GC-MS analysis of the shoot wax extract revealed the presence of more than forty compounds. n-Hexacoxanol was the major compound, representing more than 40% of the total of the wax constituents. The remaining compounds were distributed by five families: primary alcohols, n-alkanes, aliphatic aldehydes, free fatty acids, and triterpenols.

P-1184D

Acclimation and Essential Oils Characterization of Micropropagated In Greenhouse Growing Plantlets of Balm (*Melissa officinalis* L.)*. Maria C. F. B. Viana-Ferreira[1], M. J. Vilaça-Silva[2], and M. FERNANDES-FERREIRA[2]. [1]Esc. Sec. Carlos Amarante, 4700, Braga; [2]Department of Biology, University of Minho, Campus de Gualtar, 4710 - 057 Braga, Portugal. E-mail: mfferreira@bio.uminho.pt

Balm (*Melissa officinalis* L.) is a perennial subshrub which grows in tufts. Despite its low concentration (0.5 ml/g), the essential oil of this species has received most of the attention mainly due to its antimicrobial activities and spasmolytic properties. Micropropagation of this species has been accomplished from axillary buds by cultivating shoot nodal segments on solid MS medium supplemented with IBA and BAP as a multiplication medium followed by shoot transference to a rooting MS medium supplemented with IAA but devoid of pyridoxine, nicotinic acid and glycine. In greenhouse acclimation was afforded after treatment of the *in vitro* grown plantlets with a solution of Benlate and their transference to an autoclaved substratum constituted by 70% turf and 30% perlite. Substratum was maintained at 23° C with moderated watering (1 min./h) near 100% humidity, at the beginning, during the warmer days. After about three months the aerial part of the plantlets were hydrodistilled and the respective essential oils were analysed by GC and GC-MS. More than 65% of the oil was constituted by citral and the remaining part was distributed by more than thirty compounds. Apart the major compounds the essential oil profile was significantly different from that isolated from *in vivo* growing balm plants. *Project supported by Programme AGRO/ 8 /action 8.1/ project 338.

P-1185

Chlorinated Quinone and Its Related Metabolites in *Sesamum indicum* Roots and Hairy Roots. T. FURUMOTO and H. Fukui. Department of Biochemistry and Food Science, Faculty of Agriculture, Kagawa University, Kagawa 761–0795, Japan. E-mail: furumoto@ag.kagawa-u.ac.jp

Sesamum indicum L. (Pedaliaceae) is one of the most important crops throughout the world, and its seeds have been utilized for a long time as edible oilseeds and food materials. However, very little research has been reported on the constituents of the root, an unutilized part of sesame. During a search for useful and/or unique secondary metabolites in sesame roots, chlorosesamone was isolated and characterized. Chlorosesamone is a new antifungal naphthoquinone with a chlorine atom in the molecule. Natural organohalogen compounds are of increasing interest due to their frequent occurrence and various biological activities, but a limited knowledge of their biosynthetic pathway in higher plants is available. To investigate its biosynthetic pathway, we first conducted a search for metabolites biosynthetically related to chlorosesamone. Two naphthoquinones, hydroxysesamone and 2,3-epoxysesamone, and three anthraquinones were isolated from the intact sesame roots, and their structures were established by spectroscopic methods. The carbon skeletons of two naphthoquinones, a naphthoquinone ring plus a prenyl side chain, are identical with that of chlorosesamone, suggesting that these metabolites have the same biosynthetic origin. Three anthraquinones would be biosynthesized from a naphthoquinone ring plus a geranyl side chain through cyclization of the side chain. We next conducted establishment of hairy root clones by inoculation with *Agrobacterium rhizogenes* and production of the quinone compounds by hairy root cultures. One of the hairy root clones obtained was cultured in a B5 medium. Analysis of the hairy root extract showed the existence of some quinone metabolites. Therefore, *S. indicum* hairy root cultures could offer suitable system for studying the biosynthesis of chlorinated metabolites in higher plants.

P-1186

High-yield Saponin Production in Hairy Root Cultures of *Astrgalus membranaceus*. SUNG J. HWANG, Byoung S. Pyo, and Baik Hwang. Institute of Biotechnology and Department of Biotechnology, Naju 520–714. Department of Biology and HRC, Chonnam National University, Kwangju 500–757, Korea. E-mail:jinsci@lycos.co.kr

Hairy root cultures were used as a model system *in vitro* for the production of medicinally important compounds to avoid many of the problems that affect the traditional production from field-grown species. We successfully established hairy root lines of *Astrgalus membranaceus* by inoculation of aseptic stem segments with *Agrobacterium rhizogenes* ATCC15834. Transformed roots were confirmed that hairy roots examined contain both TL-DNA and TR-DNA region of Ri-plasmid by PCR amplification analysis of DNA. Hairy root lines grew rapidly with extensive lateral branching in PGRs-free medium. To optimize culture conditions for the growth of hairy roots, the type of media, carbon source, initial pH were examined. Hairy roots grow better in SH media containing 2% sucrose plus 1% glucose, pH 5.7. The highest saponin content, 5.1% dry weight, was obtained in the WPM medium supplemented with 5% sucrose. Addition of autoclaved fungal preparations or putative plant defence signalling intermediates to hairy root cultures elicited an increase in the saponin production. We have cultured hairy roots in modified airlift type bioreactor using semi-continuous culture methods. Under the optimum conditions, the maximum production of saponin reached to 5.7–6.1% dry weight after 8 weeks. This level was ca. 20 times higher in hairy root cultures than in undifferentiated cell suspension cultures.

P-1187

Production of Interleukin-2 by Hairy Roots of Carrot. SUNG J. HWANG, Byoung S. Pyo, and Baik Hwang. Institute of Biotechnology and Department of Food & Biotechnology, Naju 520–714, Department of Biology and HRC, Kwangju 500–757, Korea. E-mail: jinsci@lycos.co.kr

Due to their fast growth rates and biochemical stability, hairy root cultures remain unsurpassed as the choice for model root systems and have promise as a bioprocessing system. Applications are wide-ranging from the production of natural products and foreign proteins to model systems for plant metabolic engineering and phytoremediation. In this study, we describe the possibility that hairy root would be a good host for the production of specific protein. Interleukin-2 was originally called T cell growth factor. The major function of IL-2 in human is the activation of a variety of cell in the immune system including helper T cells, B cells, macrophages, natural killer cells, and lymphokine-activated precusors. Hairy root lines were established after the inoculation of transgenic carrot plantlets expressing IL-2 with *Agrobacterium rhizogenes* A4. For hairy root grown in MS medium, the maximum level of IL-2 protein after 4 weeks culture in shake flask was 1.67 mgl^{-1} or 0.35% total soluble protein; up to 11% of the IL-2 protein was secreted into the medium. IL-2 protein production was tested using hairy roots grown in an air-lift type bioreactor. The intracellular protein level after 8 weeks bioreactor culture was 1.2 times higher than the maximum measured in the shake flask.

P-1188

Biosynthesis of Ursane Type Triterpenes and Isolation of Fatty Acid Esters of Triterpenes from *Ternstromia japonica* Callus Tissues. IKUTA AKIRA[1] and Yoshimura Kouichi [2]. [1]Research Institute for Science and Technology and [2]Faculty of Industrial Science & Technology, Science University of Tokyo, 2669 Yamazaki, Noda, Chiba, 278–8510, JAPAN. Email: ikutaaki@rs.noda.sut.ac.jp

We have previously reported many kind of ursane type triterpenes such as ursolic acid (1), corosolic acid (2), and 3-epi-corosolic acid (3) stepwise along with biosynthetic pathway, and the characteristic 28,13β-olide triterpenes (4, 5, and 6) corresponding to the each triterpenes (1–3) from *Ternstromia japonica* (Theacea) callus tissues. We are interested in the biosynthesis of these olide derivatives and now report the tracer experiments about the biosynthetic pathway of these olide type triterpenes. The each labeled precursors (1-6) of ursane type triterpene were furnished from the extracts of the cultured cell administrated]-acetate into the culture medium of *T. japonica.* Finally, label six triterpenes were identified as pure radioactive compounds by the comparison of standard triterpenes (1–6) on HPLC and radioautography. For feeding experiments the six [U-^{14}C] compounds (1-6) were administrated separately into the culture medium of *T. japonica* callus tissues. The experiments revealed that the [U-^{14}C]-ursolic acid (1) was metabolized into 4, 2, and 3 and also the [U-^{14}C]-corosolic acid (2) was metabolized into 5, 3, and 6. In the case of administered experiments of the [U-^{14}C] ursolic acid 28,13β-olide (4) and [U-^{14}C] corosolic acid 28,13β -olide(5) into the *T. japonica* callus tissues, two unknown significant radioactive spots were detected at the Rf values indicating the presence of non-polar substances by radioautography, respectively. As the results of investigation of the constituents corresponding to nonlabeled fraction, the higher fatty acid esters of olide triterpene were obtained. This is the first report of these fatty acid esters of olide triterpene form a natural source. But the administration of 6 was not metabolized into the esters. The differences of metabolism were showed by the orientation of the hydroxy group at C-3 of the 28,13β-olide triterpenes.

P-1189

UV and Visible Radiation Effects on Flavonoid Production in Cell Cultures of *Vitis vinifera* and *Glycine max*. S. P. KOPSOMBUT, L. S. Kull, K. A. Marley, R. A. Larson, and M. A. L. Smith. University of Illinois, Department of Natural Resources and Environmental Sciences, Urbana, IL 61801. E-mail: kopsombu@uiuc.edu

Red grapes (*Vitis vinifera*) and black-seeded soybeans (*Glycine max*) are excellent resources for flavonoids, including proanthocyanidins, which have cardioprotective, antioxidant, and anticancer properties. Environmental stimuli can elicit and enhance production of bioflavonoids *in vivo* and *in vitro*. In this study, callus cultures from red grape (*V. vinifera* cv. Bailey Alicant) and both callus and cell suspension cultures derived from hypocotyl explants of black and yellow-seeded soybean (cv. Williams) were subjected to UV (354 $\mu W\ cm^{-2}$) or fluorescent (160 $\mu mol\ m^{-2}s^{-1}$) radiation either continuously or for two hours each day, over a period of two to seven days. Cultures were then maintained in the dark for seven days prior to analysis. Cell cultures were extracted with methanol and analyzed by HPLC-PDAD. Anthocyanin and proanthocyanidin content of grape callus increased dramatically with UV exposure. Neither yellow nor black soybean cell cultures produced proanthocyanidins when exposed to UV or fluorescent radiation. Yellow and black soybean cell suspension cultures produced similar concentrations of genistin, daidzin, and daidzein isoflavones. Soybean callus cultures produced a greater diversity of flavonoids compared to soybean suspension cultures, including daidzein and genistein isoflavones, and a flavone compound. Black soybeans and grapes produce bioflavonoids *in vivo*, including proanthocyanidins that are confined to seed coat tissue. UV radiation effectively stimulated proanthocyanidin accumulation in grape cell lines, but did not elicit proanthocyanidin production in soy cell lines. Proanthocyanidins appear to be confined to seed coat tissue of black soy lines, subsequent callus generation from this germplasm will rely on seed coat and cotyledonary explants, rather than hypocotyl explants.

P-1190

Conditioned Media: Identifying the Conditioning Factor and Quantifying Its Effect on Anthocyanin Production in *Vaccinium pahalae*. TRISTAN F. BURNS KRAFT1, Randy Rogers[1], David Seigler[2], and Mary Ann Lila Smith[1]. Department of Natural Resources and Environmental Sciences[1] and Department of Plant Biology[2], University of Illinois, Urbana, IL 61801. E-mail: tkraft@uiuc.edu

Conditioned media, prepared from medium filtrate that had previously supported anthocyanin-producing *Vaccinium pahalae* (ohelo) cell suspension cultures, consistently intensified the rate and level of anthocyanin accumulation in previously non-pigmented (dark-grown) cultures of *V. pahalae*, and triggered anthocyanin expression and increased cell growth in cultures of the related *V. angustifolium* (wild blueberry), which otherwise lacked pigmentation. In order to elucidate the nature of the conditioning factor, non-pigmented (dark-grown) ohelo cultures were prepared in a graded series of treatments that combined maintenance media with different percentages of conditioned media (0, 25, 50, 75, and 100). Cultures were exposed to irradiance levels of 100 $\mu mol\ m^{-2}\ s^{-1}$, 24 h d^{-1}, on a gyratory shaker at 150 rpm. At 2-day intervals for 14 days, anthocyanin content was determined using a spectrophotometer (λ = 535 nm) and fresh mass was measured. Initially, ohelo cultures had a low anthocyanin concentration (0.039 mg anthocyanin g^{-1} fresh mass), but when exposed to light, maximum increases in concentrations ranged from 40 times in 0% conditioned media to 200 times in 75% conditioned media. Cultures supplemented with a higher proportion of conditioned media began producing pigment earlier than cultures with lower proportions of conditioned media. Early in the growth cycle, cell growth rates increased as the percentage of conditioned media increased. From days 2–4, there was a 300% increase in growth rate in 75% conditioned media as compared to control (0% conditioned media). Preliminary comparison of HPLC chromatograms of conditioned versus standard maintenance media did not conclusively identify the nature of the conditioning factor. Freezing at –20°C for a period of up to 30 days and centrifugation for 6 min at 2600 rpm had no effect on the conditioning factor. Boiling for 8 minutes actually enhanced the efficacy of the conditioned media by approximately 65%, which suggests that the conditioning factor is not protein based. Dialysis experiments were conducted in an effort to determine the approximate size of the molecules or complexes that comprise the conditioning factor.

P-1191

Saponin Production from In Vitro Cell and Root Cultures of *Panax ginseng* C. A. Meyer. L. LANGHANSOVÁ, A. Nepovím, P. Maršík, and T. Vaněk. Institute of Organic Chemistry and Biochemistry, AS CR, Flemingovo nám. 2, 166 10, Praha 6, Czech Republic. E-mail: lenka@uochb.cas.cz

Panax ginseng C. A. Meyer (*Araliaceae*) is a herbaceous plant, which in oriental medicine has a strong reputation since ancient times for being tonic, regenerating, and rejuvenating. It was reported that ginsenosides and polyacetylenes isolated from ginseng roots have cytotoxic activity [1]. The current supply of ginseng mainly depends on field cultivation, which is a long and laborious process. Native ginseng plants need 5 – 7 years prior to harvest and the content of ginsenosides is low. *In vitro* mass production in large-scale systems seems to be potentially more efficient alternative for production of ginseng bioactive components. The aim of this study was to establish *in vitro* cultures producing ginsenosides identical to those in native ginseng plants. Callus and cell suspension cultures initiated from one-year-old roots of *Panax ginseng* and adventitious roots isolated from *in vitro* plantlets regenerated from somatic embryos, were compared for content of ginsenosides. Content of ginsenosides in callus culture was monitored during the year, on different modified Murashige and Skoog's media (supplemented with 2,4-D and coconut water) and under different light conditions. We established proliferous and yielding callus culture however the production ability differs during the year significantly, the content vary between 0 – 2% dr. wt. The presence of ginsenosides Rb_1, Rb_2, Rc, Rd, Re, Rf and Rg_1 were detected and quantified by HPLC and confirmed by UV spectra and retention time, and aditionaly confirmed by LC MS, in comparison with authentic ginsenosides. The maximum value of ginsenosides was found in cell suspension culture cultivated in "Aplikon" Bioreactor (Aplikon, Netherlands) with total volume of 3L, where the pure saponin content was 4,69% dr. wt. Nevertheless the saponin content in callus as well as in cell suspension cultures was limited to two major ginsenosides, Rb_1 and Rg_1. The full range of ginsenosides distributed analogous as in roots of native plants was detected in adventitious roots cultivated in liquid Schenk and Hildebrandt media supplemented with 5 mg/l IBA [2]. Moreover we founded some polyacetylen compounds. Recently we have made preliminary experiments of adventitious roots cultivation in 3L bioreactor and we are adjusting culture condition to increase yielding while preserving the ability of adventitious roots to produce whole spectrum of biologically active compounds.

P-1192

Influence of Overexpression of *Arabidopsis thaliana* Phytochelatin Synthase (AtPCS1) on Response to Cadmium Stress in Transgenic Plants. SANGMAN LEE (1), Jae S. Moon (2), Tae-Seok Ko (1), David Petro (3), Peter B. Goldsbrough (3) and Schuyler S. Korban (1). (1) Department of Natural Resources & Environmental Sciences, University of Illinois at Urbana-Champaign, Urbana, IL 61801; (2)Biopotency Evaluation Lab, Korea Research Institute of Bioscience and Biotechnology, Taejon, Korea; and (3) Department of Horticulture and Landscape Architecture, Purdue University, West Lafayette, IN 47907. E-mail:lee32@uiuc.edu

Plants produce cysteine-rich peptides such as glutathione (GSH), metallothionein, or phytochelatin (PC) to detoxify heavy metals. PCs, a family of small peptides with a general structure of (g -Glu-Cys)n-Gly (where n is 2 to 11), are enzymatically synthesized. These are rapidly induced in response to toxic levels of heavy metals in all plants evaluated for this trait. Phytochelatin synthase catalyzes the synthesis of PCs by transferring the g -Glu-Cys moiety of GSH to GSH or to other PCs. Recently, genes encoding PC synthase have been cloned from plants, yeast, and animals. We have overexpressed *A. thaliana* PC synthase (*AtPCS1*) in transgenic *Arabidopsis* in order to increase heavy metal tolerance via increased PCs synthesis. The observed increase in PC synthase levels has lead to higher sensitivity to Cd stress in some transgenic lines. The analysis and implication of this response in *Arabidopsis* will be further discussed.

P-1193

Isoflavonoids in Callus Cultures of *Pueraria lobata* (Wild.) Ohwi. ADAM MATKOWSKI. Department of Biology and Botany, Medical University in Wroclaw, Jana Kochanowskiego 10, 51–601 Wroclaw, Poland. E-mail: am9@biol.am.wroc.pl

Pueraria lobata, Fabaceae, (kudzu) root is an important crude drug used in Chinese and Japanese Traditional Medicine. Isoflavonoids are among the main active compounds, proven to be responsible for the therapeutic properties of kudzu roots. Numerous applications of this herb include: suppression of the alcohol intake by alcoholics, lowering the blood pressure and phytoestrogenic prevention of osteoporosis and other ageing-related diseases. Explants derived from both garden grown adult plants and sterile seedlings from in vitro germinated seeds. In the first case only surface sterilized stem segments were used but in young plantlets the explants were from stems, roots, leaves and cotyledons. Murashige and Skoog (MS) salts and vitamins were used as a basal medium solidified with 0.7% Bacto agar plus 2.5 g/l sucrose. Media for callus induction were supplemented with PGRs: auxins 2,4-D or NAA and the cytokinin benzyladenine (BA) in concentrations of 0.5–25 microM. Ascorbic acid 0.25 or 1 mg/l or phloroglucinol 2.5 mg/l were added for reducing phenolisation which is very strong in this species. For subcultures (8 weeks) the media with 5 or 25 microM 2,4-D and 0.5 microM BA were used. Callus growth was satisfactory in explants from young plants: roots, stems and leaves, whereas in stem segments from the adult plant the callus grew very slowly and blackened early. Isoflavonoid composition of calli was determined in methanol extracts by TLC and HPLC after third subculture. Daidzein and three glucosides were detected in the extracts from young plant calli: puerarin, 3'-methoxypuerarin and dadzin as well as small amount of genistein. In the necrotic calli from the field grown plant no convincing isoflavonoid signal was observed. Among the calli derived from different organs the highest isoflavonoid content was in root callus: puerarin 18.35 mg/100g, 3'-methoxypuerarin 25.6 mg/100g, daidzin 8.76 mg/100g (fw). In callus from other organs the amount of these compounds was considerably (three to five times) lower. Three daidzein glucosides present in callus cultures—daidzin, puerarin and methoxypuerarin are also supposed to be the most important compounds of Puerariae Radix. The results show that *in vitro* cultures of *P. lobata* can become useful for biotechnological production of phytopharmaceuticals as well as for improvement of the drug by metabolic engineering.

P-1194

Production of Anthocyanins by Hairy Root Cultures of *Ipomoea batatas*. Y. NISHIYAMA and T. Yamakawa. Laboratory of Plant Biotechnology, Dept. of Global Agricultural Sciences, University of Tokyo, Tokyo 113–8657, Japan. E-mail: aa97160@mail.ecc.u-tokyo.ac.jp

Ipomoea batatas L. cv Ayamurasaki accumulates reddish purple anthocyanins in storage roots, and it is an attractive source of natural food colorant. We attempted to make new cell lines which accumulate higher amount of anthocyanins by introducing genes of key enzymes of anthocyanin biosynthesis. We introduced phenylalanineammonia-lyase (PAL) from *Petroselium crispum* and *I. batatas* L. cv. Beniazuma, and anthocyanidin synthase (ANS) from *Ipomoea nil*. All genes were expressed under the control of *palg2b* promoter of *Populus kitakamiensis*. *ANS* was also expressed under the control of sporamin promoter of *I. batatas* L.. Transformed hairy root cultured on LS medium did not accumulate anthocyanins under continuous light irradiation. But they accumulated anthocyanins when cultured on PRL-4C medium. The effects of introduced genes were discussed.

P-1195

Production of Diterpene Alkaloids from Cultured Roots of *Aconitum japonicum*. Y. ORIHARA and K. Hattori. Graduate School of Pharmaceutical Sciences, The University of Tokyo, Bunkyo-ku, Tokyo 113–0033, Japan. E-mail: oriharay@mol.f.u-tokyo.ac.jp

Aconitum tuber is a herbal medicine used as a cardiotonic and an analgesic. Cultured roots of *Aconitum japonicum* were induced from young stems on the Murashige and Skoog's agar basal medium containing 1 mg/l of NAA and 0.1 mg/l of kinetin. The induced roots were subcultured on the same medium, and transfer into the same composition of liquid medium and cultured on a rotary shaker in the dark. Harvested roots cultured in the liquid medium were extracted with MeOH and MeOH extract was partitioned between $CHCl_3$ and aqueous ammonia. The crude alkaloid fraction was separated by silica gel column chromatography and HPLC. Five alkaloids were isolated and their structures were determined as mesaconitine (**1**), aconitine (**2**), 8-*O*-methyl-14-*O*-benzoylmesaconine (**3**), 14-acetyltalatizamine (**4**) and 15alpha-hydroxyfranchetine (**5**), by NMR and MS evidence. The time course experiments were also performed and biosynthetic studies are now in progress.

P-1196

Agrobacterium-mediated *parAt* Promotor-H6H Gene Introduction into *Duboisia* spp. L. U. RAHMAN[1], K. Amamoto[1], H. Yamamoto[1], Y. Kitamura[1], T. Muranaka[2], and T. Ikenaga[3]. [1]School of Pharmaceutical Sciences, Nagasaki University, Nagasaki 852–8521, Japan; [2]Laboratory for Biochemical Resources, Plant Science Center, Riken, Saitama 351–0198, Japan; and [3]Faculty of Environmental Studies, Nagasaki University, Nagasaki 852–8521, Japan. E-mail: laiq67@yahoo.com

Genetic transformation of *Duboisia leichhardtii* (*Dl*) and *D. myoporoides (Dm)* was established using *Agrobacterium rhizogenes* ATCC15834 with a binary vector pAH6 containing *parAt* promotor-H6H fusion gene: *parAt* promoter confers efficient statement of foreign gene in roots, and H6H (hyoscyamine-6b-hydroxylase) catalyzes the conversion from hyoscyamine to scopolamine. Transformed roots were obtained from stems and leaves of *in vitro* growing *Dl* and *Dm* shoots. Putative transformants were analyzed for the introduction of T-DNA (TR and TL regions) from Ri plasmid and foreign H6H gene from binary vector into the genome by genomic PCR. Plantlets were regenerated from a *Dl* root line containing H6H gene and TL region (A1) as well as from another root line with only TL region but not H6H gene (B0). Plants were not produced from root lines with both TR and TL regions (B2, B3, B4). The plant growth, chlorophyll content, H6H activity and alkaloid content were compared between A1, B0 and non-transformant (C). The results showed that A1 plants grew much better than B0 or C plants because of vigorous root development, whereas chlorophyll content of A1 was least among them. A1 transgenic plants contained only scopolamine and its scopolamine productivity was superior to B0 and C plants. Effect of gene introduction into *Dm* is under investigation.

P-1197

Phytoremediation of Mercury and Organomercurials via Chloroplast Genetic Engineering. OSCAR N. RUIZ and Henry Daniell. Department of Molecular Biology and Microbiology, University of Central Florida, 12722 Research Parkway, Orlando, FL 32826–3227. E-mail:daniell@mail.ucf.edu

An operon containing the *merA* and *merB* genes for the mercury degradative pathway has been targeted to the chloroplast genome of tobacco, conferring resistance to mercury and to the highly toxic organomercurials. Integration of the foreign genes coding for the mercuric ion reductase (merA) and the organomercurial lyase (merB) into the chloroplast genome occurs by homologous recombination, resulting in hyperexpression of the foreign genes because of the high copy number of the chloroplast genome per cell (up to 10,000). Stable integration of the merAB operon in the chloroplast of tobacco plants was shown by PCR and Southern blot analysis. The northern blot analysis revealed stable transcripts, independently of the presence or absence of a 3' terminator (3' UTR) on the operon. The merAB dicistron was the more abundant transcript but also less abundant monocistrons were found showing that specific processing occurs between transgenes. The expression and activity of the mercuric ion reductase and organomercurial lyase were tested through bioassays in which the transgenic plants were grown in media with different concentrations of phenylmercuric acetate and mercury chloride. The transgenic plants showed resistance to high concentrations of both toxic compounds while the wild type control died at very low concentrations of these compounds. The chloroplast transgenic plants survived higher concentrations of mercury and organomercurial than the nuclear transgenic plants containing the *merA* and *merB* genes. Therefore, chloroplast transformation is a suitable approach for bioremediation, especially when the site of action is compartmentalized within plastids.

P-1198

Novel Nicotine Alkaloid Profile in Methyl Jasmonate-elicited *Nicotiana tabacum* BY-2 Cells. SUVI SALONVAARA, Into Laakso, Tuulikki Seppänen-Laakso, Alain Goossens, Anna Maria Nuutila, Dirk Inzé, and Kirsi-Marja Oksman-Caldentey. VTT Biotechnology, FIN-02044 Espoo, Finland; Department of Plant Genetics, VIB, University of Ghent, B-9000 Ghent, Belgium; and Division of Pharmacognosy, Department of Pharmacy, FIN-00014 University of Helsinki, Finland. E-mail: suvi.salonvaara@vtt.fi

Methyl jasmonate (MeJA) is known to induce the accumulation of numerous defence-related secondary metabolites (e.g. phenolics and alkaloids) through the induction of genes coding for the enzymes involved in the biosynthesis of these compounds in plants. In our project, the formation of various nicotine related alkaloids in tobacco BY-2 cells was studied after elicitation with MeJA. Identification and quantification of these alkaloids was performed by GC-MS from samples taken at several time points during a period of 98 hours. The accumulation of alkaloid metabolites in the cells started after 14 hours and reached their maximum levels towards the end of the experimental period. The main components were anatabine and two isomers of anatalline, while anabasine and nicotine were present as minor compounds only. Transcript profiling based on cDNA-AFLP indicated that almost one third of the MeJA-induced genes are putatively novel genes some of which are possibly involved in nicotine alkaloid biosynthesis.

P-1199

Higher Levels of $ZnSO_4$ and $CuSO_4$ Enhance Secondary Metabolite Contents in Cultures of *Ammi majus* and *Psoralea corylifolia*. P. S. SRIVASTAVA and D. Pande. Centre for Biotechnology, Faculty of Science, Hamdard University, Hamdard Nagar, New Delhi 10062, India. E-mail: root@hamduni.ren.nic.in

Phytotoxicity of heavy metals due to industrial pollution has serious implications. Heavy metals differ in their role in metabolic functions. Generally, low quantity of zinc and copper are essential heavy metals for higher plants and act as a cofactor or a part of prosthetic groups of enzymes in a wide variety of metabolic and developmental pathways. At higher concentrations, however, they become toxic and hamper plant growth. Plant tissue culture is an excellent technique to raise metal tolerant plants as it provides a convenient way of generating useful variations in the cultured population. *Ammi majus* (Apiaceae) and *Psoralea corylifolia* (Fabaceae) are rich sources of natural xanthotoxin and psoralen, respectively. These compounds are commonly used in the treatment of leucoderma and leprosy. The present study reports the effect of various levels of $ZnSO_4$ and $CuSO_4$ on morphogenic, biochemical and phytochemical responses of *Ammi majus* and *Psoralea corylifolia*. Incorporation of $ZnSO_4$ (100 μM) to MS + IAA + Kn + Ad + CH promoted growth and accumulation of higher amounts of xanthotoxin (14.8 mg/g dw) than controls (10.6 mg/g dw). Enhanced amounts of psoralen (18.3 mg/g dw) was observed in cultures on MS + IAA + Kn + Ad + Asp + Glu + CH + 200 μM $ZnSO_4$. In control cultures it was 9.4 mg/g dw. *A. majus* could tolerate $CuSO_4$ upto 50 μM and yielded 11.9 mg/g dw of xanthotoxin. Whereas, *P. corylifolia* could tolerate $CuSO_4$ upto 100 μM, showed early flowering and an enhanced amount of psoralen (24.6 mg/g dw). Higher concentrations of $ZnSO_4$ and $CuSO_4$ affected morphogenic responses and secondary metabolite yield. Proline and protein accumulated as a sequel to metal stress.

P-1200

Biosynthesis of Abietane Diterpenoids in Cultured Cells of *Torreya nucifera* var. *radicans*: Biosynthetic Inequality of the FPP Part and the Terminal IPP. J. YANG and Y. Orihara. Graduate School of Pharmaceutical Sciences, The University of Tokyo, Bunkyo-ku, Tokyo 113–0033, Japan. E-mail: ff07018@mail.ecc.u-tokyo.ac.jp

Abietane diterpenoids are a class of natural products widespread in the plant kingdom. We isolated the abietane diterpenoids, such as hinokiol, from the cultured cells of *T. nucifera* var. *radicans*. Recently, a totally different pathway from the former acetate/mevalonate pathway on the biosynthesis of isoprenoids, in which the basic C_5 unit, isopentenyl diphosphate (IPP), is formed from glyceraldehyde 3-phosphate (GAP) and pyruvate, was discovered in bacteria, green alga and higher plants. However, the pathway utilized for the formation of the IPP unit in the abietane diterpenoids has not yet been clarified. Therefore, we administered [1-^{13}C] glucose, [1-^{13}C] sodium acetate, [1,2-$^{13}C_2$] sodium acetate and [1-^{13}C] sodium acetate+glucose into the two-week-old suspension culture of *T. nucifera* var. *radicans*. After one week incubation, the fresh cells were harvested and extracted with MeOH. The MeOH extract was partitioned between EtOAc and H_2O. The EtOAc extract was subjected to silica gel and the subsequent purification by C_{18} HPLC resulted in the isolation of ^{13}C labelled hinokiol. It was proved that the IPP unit in the abietane diterpenoids were biosynthesized via the GAP/pyruvate pathway, as well as the acetate/mevalonate pathway through the labelling experiments with [1-^{13}C] glucose, [1-^{13}C] sodium acetate and [1,2-$^{13}C_2$] sodium acetate. In addition, the terminal IPP showed the preferential labelling from the GAP/pyruvate pathway and unfavorable labelling from the acetate/mevalonate pathway, suggesting that the biosynthetic route leading to the terminal IPP involved the GAP/pyruvate pathway more and the acetate/mevalonate pathway less, compared with the FPP part. Furthermore, it was also found that the utilization of the two pathways in the formation of the FPP part and the terminal IPP was dependent on the exogenous precursors through the simultaneous administration of [1-^{13}C] sodium acetate and unlabelled glucose.

P-1201

Production of Secondary Metabolites from *Glycyrrhiza glabra* Hairy Root Cultures. T. YOSHIKAWA, Y. Asada, W. Li *, and T. Nikaido*. School of Pharmaceutical Sciences, Kitasato University, 5–9–1 Shirokane, Minato-ku, Tokyo 108–8641, Japan and *School of Pharmaceutical Sciences, Toho University, 2–2–1 Miyama, Funabashi-city, Chiba 274–8510, Japan. E-mail: yoshikawat@pharm.kitasato-u.ac.jp

In our studies on hairy root cultures to produce useful compounds, we investigated the constituents of *Glycyrrhiza glabra* hairy root cultures which were established by induction of Agrobacterium rhizogenes pRi 15834; pBI 121(GUS). Although a large amount of the crude saponin fraction was prepared from the hairy root liquid cultures and several saponins were isolated from *G. glabra* hairy root culture, glycyrrhizin, the main saponin of some *Glycyrrhiza* species was not detected in its cultures. From the same saponin fraction some triglycosides such as soyasaponin I and II, having sophoradiol, soyasapogenol B and E as the aglycon were isolated at a high yield from its cultures. On the other hand, the ethyl acetate extract was chromatographed on a silica gel column and purified by normal-phase HPLC to give 35 flavonoids including 11 new compounds. The isolated flavonoids had various structures such as chalcone, dibenzoylmethane, retrochalcone, flavanone, isoflavan, isoflavone and aurone. Two dimeric flavonoids and many prenylated flavonoids were isolated from its cultures as the characteristic constituent of *G. glabra* hairy root. The glycosidic fraction was applied to ODS column chromatography and purified by reverse phase HPLC to give 11 glycosides including 2 new compounds. It is very interesting that the isolated flavonoids could be obviously divided into two groups in the course of their biosynthesis. The flavonoids lacking 5-hydroxyl substituent are biosynthesized to isoflavones, then further glycosylated to afford *O*-glucosides, while the flavonoids possessing 5-hydroxyl substituent are biosynthesized to flavone *C*-glycosides. The reason for this are not clear and need further study.

P-1202

The Role of Light and Temperature Regulating Anthocyanin Production in Cranberry Callus. YU ZHOU and Bal Ram Singh. Department of Chemistry and Biochemistry, and The School for Marine Science and Technology, University of Massachusetts Dartmouth, North Dartmouth, MA 02747. E-mail: YZHOU@UMASSD.EDU

In order to find out the role of light and temperature regulating anthocyanin production at cranberry cell level, cranberry callus was successfully induced from stems of cranberry (*Vaccinium macrocarpon* Ait, Ericaceae) by using Gamborg' s B5 medium containing NAA, 2,4-D and kinetin at 25°C in the dark. Anthocyanin-producing cranberry callus was observed under continuous light exposure. Cranberry callus was cultured under continuous light exposure at 25°C for three weeks then transferred to 15°C under same continuous light exposure for one week. The change of temperature from 25°C to 15°C resulted in increased anthocyanin production in cranberry callus. In order to evaluate the role of temperature, the callus treated with 15°C was transferred to 30°C, 37°C, and 42°C in the dark. Another batch of callus was transferred to 4°C, and randomly divided to three groups: one group received no light, second group received red light, and third group received UV light. The callus was cultured under above conditions for 48 hours. 0.25 gram of each sample was weighted, and homogenized in 1.25 ml of a mixture of 95% ethanol: 1.5 N HCl = 85:15 v/v to extract the anthocyanins overnight at 4°C. Anthocyanin contents of each sample were quantitatively determined by UV/VIS Spectrophotometer at 535 nm. The light-dependent anthocyanin production in cranberry callus was regulated by temperature. Anthocyanin contents in cranberry callus cultured at different temperatures in the dark were decreased 81.1% at 42°C, 58.9% at 37°C, 47.0% at 30°C compared to the callus at 25°C. Red light stimulated anthocyanin accumulation by 25.8%, in comparison to those kept in the dark.

P-1203

Physical and Genetic Mapping of Transgenes in Barley. LORELEI J. BILHAM, Silvia Travella, Haroldo Salvo-Garrido, John W. Snape, and Wendy A. Harwood. Crop Genetics Department, John Innes Centre, Norwich Research Park, Colney, Norwich, NR4 7UH, UK. E-mail lorelei.bilham@bbsrc.ac.uk

The most important part of the transformation process over which, at present, we have no control is the site of transgene integration. It is this area we are working to address by developing and applying methodologies to determine the physical and genetic location of transgenes in barley. Fluorescent *in situ* hybridisation (FISH) was used to locate transgenic DNA on metaphase chromosomes by hybridisation of a labelled probe, made from copies of the introduced plasmid. Characterised rDNA markers were used to give distinct and specific chromosome patterns to unambiguously identify each barley chromosome. FISH was used as a starting point for fine intrachromosomal genetic mapping using Restriction Fragment Length Polymorphisms (RFLP) and Simple Sequence Repeat (SSR) markers to determine the genomic map location of the transgenes. The physical location of transgenes has been determined at the chromosomal level by FISH in 17 independent transgenic barley lines. Genetic mapping has confirmed the genomic location of transgenes in 8 of these lines. Our results suggest that in barley, transgene integration may not be random since sites of transgene insertion were found in only 4 of the 7 barley chromosomes.

P-1204

cDNA Microarray Analysis of Hot Pepper (*Capsicum annuum.* L) During Fruit Ripening Process Using 4,320 cDNA Clones. J. S. CHOI, J. Y. Park, H. J. Kim, W. Y. Song, B. J. Oh, C. O. Lim, and J. C. Hong. Department of Molecular Biology and Biochemistry, Gyeongsang National University, 660–701, Korea. E-mail: jchong@nongae.gsnu.ac.kr

Pepper (*Capsicum annuum*) fruit development and maturation is characterized by activation of key metabolic pathways leading to fruit pigmentation, enhanced flavor, and protection against various biotic and abiotic stress. Some of the of newly formed mRNA. To study temporal changes in mRNA level during pepper fruit ripening changes are due to expression microarray technology was employed using 4,320 cDNAs from libraries of red pepper pericarps spotted in high density on glass microscope slides. Patterns of gene expression in green pepper and red pepper were compared using fluorecently labeled mRNA population. The 423 cDNA clones that respond to fruit ripening were selected, sequenced and further analyzed. cDNA clones that were found to represent majority of mRNA are genes encoding plant (1)fibrillin, (2)gamma thionin, (3)capsanthin synthase, and many genes related to plant defense and environmental stress. In addition, genes involved in transcription, secondary metabolism, cell wall metabolism, and signal transduction were also identified. This study shows the relevance of microarray technology in identifying many argonomically important genes. (This work was supported by BK21 program and the grant from PDRC of 21C Frontier Research Program)

P-1205

Expression of a Modified Ac-Transposase in Barley (*Hordeum vulgare* L.). CELIA K. FRIEDRICH, Horst Loerz, Stephanie Luetticke. Centre for Applied Plant Molecular Biology, University of Hamburg, D-22609 Hamburg, Germany. E-mail: C_Friedrich@botanik.uni-hamburg.de

Transposable elements are mobile genetic units that can mutagenise genes by insertion into coding and regulatory genomic regions. The transposable element *Ac* from maize (*Zea mays* L.) encodes the trans-acting enzyme transposase (TPase) and has cis-acting subterminal and terminal sequences. Induction and stabilisation of transposon generated mutations is possible by separating the cis- and trans-acting parts of the *Ac* sequence in a two-component-system: a mobile non-autonomous element and a *TPase*-gene. An *Ac*-based two-component-system has been established in barley (*Hordeum vulgare* L.). The employed modified TPase has been previously shown to mediate higher transposition activity than the wild-type TPase. To achieve a better understanding of the TPase regulation the number of transgene copies was determined and *TPase*-transcripts were detected in several transgenic barley lines of the two-component-system. All lines have the correctly spliced and an alternatively spliced *TPase*-transcript. One line with detectable amounts of unspliced transcript showed higher TPase-activity in *TPase*-expressing scutellum tissue. The TPase-activity was assayed by reportergene detection on *TPase*-expressing tissue biolistically transformed with a construct containing a non-autonomous element flanked by the *actin1*-promoter at the 5' and the uidA-gene at the 3'-end. In addition analyses of plants carrying both components show that in barley the expressed TPase is able to activate non-autonomous-elements *in planta*. The effects at different stages of *TPase*-expression for example the amount of transcript and functional TPase in barley are discussed.

P-1206

Male Specific RAPD and SCAR Markers in Hemp (*Cannabis sativa* L.). Hajnalka Homoki[1], Nándor Bucherna[1], Ottó Törjék[1], Erzsébet Kiss[1], Zsuzsanna Finta-Korpelová[2], Iván Bócsa[2], István Nagy[3] & LÁSZLÓ E. HESZKY[1]. [1]Dept. of Genetics & Plant Breeding, St. István University, Gödöll, Hungary, 2103; [2]Agricultural Research Institute, St. István University, Kompolt, Hungary; and [3]Agricultural Biotechnology Center, Gödöll, Hungary. E-mail: heszky@fau.gau.hu

Twenty decamer RAPD primers producing sex-specific markers in different plant species were tested on some Hungarian hemp cultivars. Two of them (OPD05 and UBC354) generated male-specific markers. These two DNA fragments were isolated, cloned and sequenced. Both markers are unique, because there is no sequence with significant homology to $OPD05_{961}$ and $UBC354_{151}$ markers in sequence databases. Male-specific RAPDs were converted to SCARs. These SCAR markers ($SCAR_{323}$ and $SCAR_{119}$) were efficient in identification of all male plants and were able to amplify a single DNA band in both cases. SCAR markers were used to analyse randomly chosen F_2 plants. The SCAR markers correlated to the sex of the segregating population, except 2 male plants where these markers were missing. Results of plant analysis of F_2 population suggest these markers are to be linked to the Y chromosome. This research was supported by the OTKA Grant (T 030774).

P-1207

The Use of RAPD Markers for Fingerprinting and Genetic Diversity Assessment in Portuguese Tea Plants (*Camellia sinensis* (L.) O. Kuntze). S. JORGE*, G. Brown**, D. B. Neale**, and M. C. Pedroso*. *Faculdade de Ciências da Universidade de Lisboa, Departamento de Biologia Vegetal, Centro de Biotecnologia Vegetal, Bloco C2, Piso 1, Campo Grande, 1749–016 Lisboa, Portugal, and **Department of Environmental Horticulture, University of California, Davis, CA 95616. E-mail: smsj@fc.ul.pt.

Random amplified polymorphic DNA (RAPD) markers were used for fingerprinting and to assess genetic diversity among the Portuguese *Camellia sinensis* (tea plant) accessions and those obtained from the germplasm collections from Tea Research Foundation of Kenya and from the National Research Institute of Vegetables, Ornamental Plants and Tea of Japan. The accessions studied are taxonomically classified as belonging to *C. sinensis*, *C. assamica* and *C. assamica* ssp. *lasiocalix*. A set of 118 10-base-long arbitrary primers was tested, of which 25 produced informative, reproducible and polymorphic band pattern. These primers were used to amplify 71 tea plant accessions and produced 282 polymorphic RAPD markers. Our study shows that the accessions studied, including the Portuguese tea plants, exhibit considerable genetic variability. From the UPGMA cluster analysis, it was possible to distinguish the Portuguese tea plants from the remaining accessions. The RAPD markers discriminated the three-*C. sinensis* taxonomic varieties. Moreover, within each variety cluster, sub-clusters formed according to their geographic origin, i.e. within *C. sinensis* var. *sinensis* was possible to distinguish the plants obtained from Japan and those from China. In conclusion, RAPD markers are phenetically informative and represent a suitable method to estimate genetic variability in *Camellia sinensis*. RAPDs data provided a high degree of resolution at the variety level and strongly suggest that genetic differentiation be based on variety type and also on geographical distribution. This work was supported by the Foundation for Science and Technology (Lisbon, Portugal), through a fellowship to S. Jorge (PRAXIS XXI/BD/13870/97).

P-1208A

Microarray Analysis of Gene Expression in Soybean Roots Attacked by the Soybean Cyst Nematode. B. F. MATTHEWS, R. Khan, N. Alkharouf, Hunter Beard, M. MacDonald, and Halina Knap. USDA, ARS, Soybean Genomics & Improvement Laboratory, BARC-West, Beltsville, MD 20705. E-mail: MATTHEWB@BA.ARS.USDA.GOV

The soybean cyst nematode (SCN) is the major pest of soybean in the US and causes an estimated one billion dollars worth in damage each year. The defense response of soybean to SCN is a multigenic trait and varies depending upon the genotypes of soybean and SCN. The expression of over 3,000 soybean genes was monitored using microarrays to identify genes involved in the response of soybean to SCN. RNA was harvested from roots of soybean cv. Peking resistant to SCN race 3 and cv. Kent susceptible to SCN race 3, either not infected or at several time points after infection by SCN race 3 and was fluorescently labeled as cDNA for hybridization to the microarrays. A number of defense-related genes were at least 2-fold induced in the presence of SCN. Among the genes induced in soybean roots are defense-related genes, genes encoding potential regulatory factors such as phosphatases and transcription factors, and a number of genes encoding proteins of unknown function. These results provide some insights into the mechanisms used by soybean to respond to SCN attack. See our web site at http://bldg6.arsusda.gov/benlab/ for further information.

P-1208

Genomic Approach to Characterize Gene Loci Coding Wheat Storage Proteins and Starch Biosynthesis Enzymes. Y. Q. GU, X. Kong, D. Coleman-Derr, R. Chibbar, G. Lazo, F. You, and O. D. Anderson. Genomics and Gene Discovery Unit, USDA-ARS-WRRC, 800 Bucahnan St., Albany, CA 94710. E-mail: ygu@pw.usda.gov

Wheat endosperm is a storage tissue that is mainly comprised of storage proteins and starch, which are what make wheat a staple food for humankind. The genes encoding the seed storage proteins and starch biosynthesis enzymes are major determinants of wheat quality, which is directly measured by breadmaking ability and nutritional value. An understanding of the structure and expression of these genes is essential for genetically engineering improvement of wheat quality. In this study, we screened a cultivated tetraploid wheat (Triticum turgidum var. durum) BAC library using 4 seed storage protein probes and 8 starch biosynthesis enzyme probes. Between 4 and 40 positive clones were obtained for each of the single-copy starch biosynthesis genes. A total of 223, 56, and 92 positive clones were recovered for the ?-gliadin, ?-gliadin and low molecular weight glutenin (LMW-glutenin), respectively, which are known to belong to large gene families. Agarose gel-based fingerprinting analysis was performed on the a- and g-gliadin BAC clones. The data were collected and used in the FPC program to assemble contigs that span the large loci of gliadin gene families. The validity of these ordered BAC contigs were further verified by Southern hybridization using several specific probes. Twenty-three positive clones carrying HMW-glutenin genes were obtained from the screening. The high molecular weight glutenin genes (HMW-glutenin) are divided into similar x-type and y-type paralogous genes which evolved by duplication. Fingerprinting analysis was performed to assembly BAC contigs for the HMW-glutenin loci. Two contigs were resolved from the analysis, each carrying the x-type and y-type HMW-glutenin genes, one from the A and the other from the B genomes. The sequencing of HMW-glutenin BAC clones is underway to determine gene content and order, as well as sequence variation in the flanking regions of the two types of HWM-glutenin genes. The results from the BAC sequencing will be discussed. Characterization of BAC contigs for loci carrying seed storage protein genes will enable us to determine their complexity in the wheat genome.

P-1209

High Throughput, 96-well-uniplate Format for Extraction of Plant Genomic DNA—Produces DNA Suitable for Restriction Digestion, Southern Analysis and PCR. P. D. MATTHEWS*, Jesse R. Christenson, Charlie D. Gustus, and David A. Somers. *S. S. Steiner, Inc. 655 Madison Avenue, NY, NY and Agronomy and Plant Genetics, University of Minnesota, St. Paul, MN 55108.

To allow high throughput (384/day) analysis of genomic DNA from small amounts of plant tissue, a standard nucleic acid extraction was formatted to multi-welled uniplates by linking a novel series of interlocking plates. The protocol incorporates steel pellet tissue homogenization as well as unique liquid handling and phase separation techniques. Phase Lock Gel™ (Brinkmann/Eppendorf) used on a 96-well block allows phenol/chloroform extractions in parallel. The implementation is inexpensive and requires minimal special equipment, including a paint shaker and a uniplate centrifuge rotor. The extraction buffer may be modified to suit particular plants and other nucleic acids (RNA). Cost calculation from a materials and suppliers detail presented is US $0.18 per sample. The method is validated for difficult plant DNAs with representative agarose gel separations of restriction fragments and Southern blot analysis T0s in soybean. The range of success rates for soybean Southern blots is 73 (70/96) – 92 (94/96) % per sample. Variation in absolute yield was quantified by two independent means: (1) densiometric image analysis of ethidium bromide-stained agarose gels and (2) microplate flourimetry. The intersample absolute yield variation per plate is small enough to obviate individual sample concentration adjustment prior to Southern analysis. Average absolute yield for the barley mapping population based on flourimetry was 6.8 ug +/- 0.85 S.E.M, n =96; providing enough pure, stable DNA for many individual PCR reactions. Intersample cross-contamination and suitability for PCR analysis was validated by SSR display of previously characterized barley and hop mapping populations. Molecular marker displays from the uniplate method and from an individual sample preparation method are presented for comparison. Although the protocol requires some skill and practice, we find it to be a robust, adaptable and versatile home-brewed alternative.

P-1210

Improvements in Soybean Transformation Using the *Agrobacterium*-mediated Cotyledonary-node Method. P. M. OLHOFT, L. E. Flagel, C. M. Donovan, and D. A. Somers. Agronomy and Plant Genetics, 1991 Upper Buford Circle, 411 Borlaug Hall, University of Minnesota, St. Paul, MN 55113. E-mail:olhof003@tc.umn.edu

The success of soybean EST sequencing projects has increased the demand for functional genomics strategies based on efficient transformation systems for the production of transgenic plants. We have improved the soybean cotyledonary-node method, which uses Agrobacterium tumefaciens to deliver T-DNA into axillary meristematic cells through the addition of L-cysteine and other thiol-containing compounds to the solid co-cultivation medium and development of a new selection regime. Addition of L-cysteine increased Agrobacterium infection and T-DNA transfer in the cotyledonary-node region from 37% in control explants to 91% of explants after five days co-cultivation and resulted in a fivefold increase in stable transformation of shoot primordia. Selection of transformed cells during shoot initiation and elongation employing hygromycin B resistance significantly improved regeneration of transgenic shoots with almost no non-transgenic escapes compared to other selective agents tested. The efficiency of transformation measured as the number Southern-positive fertile plants produced per explants inoculated was increased from a level of 0.7%, achieved using the standard method, to an average of 7%. Enzymatic browning was reduced on the thiol-treated explants, suggesting that thiol-containing compounds suppress wound and pathogen-defense responses in soybean cotyledonary-node cells thereby increasing transformation efficiency.

P-1211

Abstract has been withdrawn

P-1212

Interaction between Maternal and Filial Tissues in the Developing Barley Grain: an Approach in Functional Genomics. V. V. RADCHUK, N. Sreenivasulu, L. Altschmied, U. Wobus, W. Weschke. Institute of Plant Genetics and Crop Plant Research (IPK), Correnstrasse 3, D-06466 Gatersleben, Germany. E-mail: radchukv@ipk-gatersleben.de

The developing cereal grain consists of three genetically different tissues, the maternal pericarp and the filial endosperm and embryo. Whereas most of the published research has devoted to the filial tissue, interactions between the maternal and the filial parts of the grain in the early development are poorly understood. We have analysed spatial and temporal mRNA profiles in the developing maternal and filial parts of the barley grain during the pre-storage (0–5 days after flowering, DAF) and the initial storage phase (6–12 DAF). A high density cDNA macroarray filter containing about 1440 PCR-amplified unigene EST fragments mainly from young developing caryopses was hybridised to ^{33}P-labeled cDNA probes derived from dissected embryosac and pericarp tissues in two-day intervals (from 0 to 12 DAF). After normalisation of the hybridisation signals, K-mean cluster analysis was applied based on standard statistical algorithms to arrange genes according to similar expression patterns. As a result, six solid clusters were revealed with sets of genes preferentially expressed in pericarp or embryosac. During the pre-storage phase, most of the caryopsis consists of the maternal tissue, which serves as transient storage tissue mainly for starch and provides the early endosperm/embryo with nutrients. In this stage, most upregulated in the pericarp genes encode transport- and carbohydrate metabolism-associated transcripts, whereas genes preferentially expressed in the embryosac are mostly related to cell division, elongation and encode regulatory proteins as well. During the early storage phase (6 DAF onwards), up-regulation of genes encoding the key enzymes of starch synthesis, ADP-glucose pyrophosphorylase, sucrose synthase and granule bound starch synthase, was noticed in the embryosac. At the 8–10 DAF, the storage protein transcripts were upregulated in the filial tissue, indicating the beginning of protein accumulation within the aleuron layer and the starchy endosperm. As expected, housekeeping genes are expressed in both the maternal and the filial tissues to nearly the same level. Northern blot analyses, in situ hybridisations and physiological studies perfectly confirm and complement the macroarray data. The presented cDNA macroarray analysis provides the possibility to analyse the tissue-specificity of biochemical pathways on the transcript level.

P-1213

An Evolutionary History of the 14–3–3 Protein Family in Plants. M. F. REYES & R. J. Ferl. Department of Horticulutral Sciences, University of Florida, Gainesville, FL 32611. E-Mail:matt@astro.ufl.edu

The 14–3–3 proteins are a highly conserved family of proteins that participate in signal transduction primarily by binding specifically phosphorylated proteins. Multiple isoforms have been detected in every eukaryote in which 14–3–3's have been investigated. These results both permit and prompt the examination of this protein family's evolutionary history. Using specialized bioinformatics tools to analyze the isoforms present in Arabidopsis thaliana and in other plant species yields insight on what the ancestral plant 14–3–3 sequence was and what its function might have been. Structural information is used to show evolutionary trends and to "modularize" amino acid and DNA sequences for individual examination. Best fit phylogenetic trees are also created from these modules and evolutionary distances and dates are calculated based on the ratio of non-silent mutations to silent mutations. This computational approach requires the consideration of both DNA and amino acid sequences. This knowledge can guide future research into 14–3–3 interactions with other entities in vivo. Future evolutionary studies will also include the many 14–3–3 proteins present in animal species as well as in other eukaryotes.

P-1214

Gene Expression Profiling in Cells of the Female Gametophyte of Wheat (*Triticum aestivum* L.) Before and After Fertilization. STEFANIE SPRUNCK, Neil Shirley*, Ute Baumann*, Peter Langridge*, Thomas Dresselhaus. Applied Plant Molecular Biology II, University of Hamburg, Ohnhorststrasse 18, D-22609 Hamburg, Germany and * Dept. Plant Science, University of Adelaide, Waite Campus, Waite RD, S.A. 5064, Australia. E-mail: sprunck@botanik.uni-hamburg.de

The ovule of seed plants represents the female reproductive organ consisting of highly differentiated structures: integuments surround the nucellus tissue and eventually become the seed coat. Within the nucellus, the female gametophyte (embryo sac) develops during the process of megasporo- and megagametogenesis from a haploid megaspore. In wheat, the mature embryo sac consists of the egg cell (gamete) which develops into the embryo after fertilization, two synergids which might play an important role in pollen tube guidance, a central cell which forms the endosperm after fertilization and some antipodal cells. The developmental processes responsible for embryo sac formation and following fertilization are based on differential gene expression and are of crucial importance for wheat grain development and grain quality. We are using a functional genomics approach which allows the parallel and comparative analysis of expression pattern of many genes to identify candidates which are preferentially expressed within the different cell types of the wheat embryo sac. In addition, we are studying genes which are up- or down regulated early after fertilization. Applying an experimental system to microdissect embryo sac cells of wheat, we are able to isolate egg cells, synergids and central cells from unpollinated ovules. Furthermore, we isolate early zygotes (2-nucleoli stage) and 2-celled and 4-celled proembryos after cultivation using a feeder system. cDNA populations can be generated after mRNA extraction out of 10–20 cells each. Different strategies are now being applied to analyze the obtained cDNAs: cDNA libraries were constructed for profiling gene expression via sequencing some 1,000 ESTs each. Transcript analysis of different cell types was further performed by Restriction Fragment Differential Display (RFDD) PCR. Additionally, Suppression Subtractive Hybridization (SSH) was performed with cDNA populations of whole ovules bevore and after fertilization. EST sequencing of the first libraries has been started and the first results from data base analyses. RFDD-PCR and SSH will be presented.

P-1215

Transposon-mediated Gene Tagging in a Small Grain Cereal (*Triticum monococcum*). S. TRAVELLA and B. Keller. Institute of Plant Biology, University of Zürich, Zollikerstr. 107, CH-8008 Zürich, SWITZERLAND. E-mail: stravella@botinst.unizh.ch

The identification of agronomically important genes remains the big challenge for application of biotechnology in wheat. Transposon mutagenesis is a powerful technique for isolating genes that encode unidentified products in a variety of organisms. The best characterised transposons of plant origin are the Ac- and Ds- elements and the En/Spm transposon of maize. Studies in the diploid plant *Arabidopsis* have indicated that transposons of the Ac-, Ds- origin tend to integrate into loci that are genetically linked to the previous site of integration, whereas the En/Spm transposon tends to transpose more often to unlinked positions in the genome, showing a fairly even distribution over the genome. We have developed such a transposon mutagenesis system in the diploid wheat *Triticum monococcum*, which should ultimately allow the tagging of agronomically important genes in wheat. We have used the En/Spm transposon system for transformation of *T. monococcum*. Being diploid, *T. monococcum* allows the investigation of a mutation in homozygous mutant plants without the presence of orthologous genes that might compensate for the mutation. So far, no reports concerning *T. monococcum* transformation were published. The transformation procedure of *T. aestivum*, which is well established in our laboratory based on mannose selection, was used to transform *T. monococcum*. *T. monococcum* was transformed using microprojectile bombardment of immature embryos with plasmid DNA containing the En/Spm transposon. Regenerating plants were checked out for integration of the entire transposon-coding sequence. In subsequent generations, the mobility of the transposon will be followed. Such a gene knockout system will provide the scientific community with cereal plants containing mutations in genes and will thus provide valuable tools for the identification of gene functions that are specific for small grain cereals.

P-1216

Establishment and Application of a Tomato Microarray for Evaluating Expression Levels in Solanaceae (sps.). EWA URBANCZYK-WOCHNIAK, Lothar Willmitzer, and Alisdair R. Fernie. Max Planck Institut für Moleculare Pflanzenphysiologie, Am Mühlenberg 1, 14476 Golm, Germany. Email: urbanczyk@ mpimp-golm.mpg.de

We intended to establish a system, which would have enabled detailed studies of gene expression in different Solanaceaeous species. For this purpose, we have constructed a cDNA microarray containing approximately 2000 tomato (Lycopersicon esculentum) ESTs. The chosen ESTs corresponding to 1000 essential genes mainly involved in primary metabolic pathways. These arrays have been hybridised in series of experiments with probes derived during different developmental stages of the potato tuber. Transgenic potato lines, exhibiting enhanced sucrose mobilization were analyzed (Roessner et al., 2002). These lines display dramatically reduced sucrose levels and have previously been thoroughly characterized by the use of metabolic profiling – where they were found to be very distinct from wild type tubers. Results from the hybridization experiments will be discussed with particular attention being paid on identifying changes in gene expression that can help in the understanding of the mechanisms by which carbon partitioning is altered in these lines.

P-1218

Activation of Mobile Elements Through Tissue Culture in Rye (*Secale cereale* L.). A. M. VAZQUEZ, I. Ballesteros, E. F. Alves, R. Linacero. Departamento de Genética, Facultad de Biología, Univ. Complutense, 28040 Madrid, Spain. E-mail: anavaz@bio.ucm.es

It is know that different stresses could exert a mutation pressure over the genome and induce changes through different mechanisms. Plant tissue culture has been pointed out to be one of these stresses and the genetic variability generated is known as somaclonal variation. We performed RAPD analysis to assess DNA variation among rye in vitro regenerated plants.Through the study of the amplification patterns several mutations, expressed by the modification of some bands, were observed. We identified several hypervariable bands, modified in plants with different origins, which represent hot spots of DNA instability in the rye genome (Linacero et al.,TAG 100: 506–511, 2000). We study these bands in order to know the sequences involved in these variation as well as the mechanism implicated in their appearance. Some of these sequences were related with mobile elements and we were able to prove in three cases that activation of these elements had been promoted. The F20 band could be explained by a new insertion of a BARE-1-like retrotransposon. The C20a and C20b bands clearly indicated that a transposon-like element was inserted or excised. It is surprising that these mobile element enters in the same place in different plants. Finally the appearance of the F13ab band could be related with the amplification of a gypsy retrotransposon. Tissue culture could be an important tool to study, at least in rye, the mobile elements.The high rate of activation as well as the possible preferential target of insertion or elimination of these elements represent an open field of research which could help in the knowledge of the mechanisms generating the high genetic variability which this species shows.

P-1219

The Genomic Sequence and the Gene Copy Number of the Peanut Allergen Ara H 2 Confirm It as a Doublet. O. M. VIQUEZ, K. N. Konan, and H. W. Dodo. Department of Food and Animal Sciences, Food Biotechnology Laboratory, Alabama A&M University, Normal, AL, 35762. Email: oviquez@aamu.edu

Peanut allergy is a crucial problem of national importance affecting millions of Americans children and adults. Peanut Allergens are seed storage proteins, Ara h 2 is a major allergen triggering allergic reactions In over 90% of the peanut allergic population. When a protein profile of a crude peanut extract is run on a SDS-PAGE, allergen Ara h 2 migrates as two bands of approximately 17 kDa. The objectives of this research were 1) to clone and sequence peanut allergen Ara h 2 at the genomic level and, 2) to determine its gene copy number. A peanut genomic library was screened using a ^{32}P-labeled 80bp oligonucleotide probe constructed based on the Ara h 2 cDNA sequence. Southern hybridization of an EcoR I digested peanut genomic DNA was performed using a 400bp PCR fragment of the genomic clone as a probe to determine the gene copy number of allergen ara h 2. Sequence information revealed a full-length genomic clone for peanut allergen gene Ara h 2, with an ORF of 621bp and a deduced 207 amino acid residues. A comparison of the genomic and cDNA sequences revealed 100% homology up to nucleotide 471 after which the two clones diverge revealing the presence of at least two isomers of the Ara h 2 gene. The Ara h 2 gene copy number reveals two EcoR I bands at position 2 kb and 5 kb. The combined results of the genomic and cDNA sequence, and the data from the gene copy number, confirm the presence of two Arah 2 gene copies in the peanut genome.

P-1220

Agrobacterium-mediated Transformation of Elite Public Soybean Genotypes for Soybean Functional Genome Study. Peiyu Zeng, Jamie Schlereth, Lan Wang, Joe Polacco, and ZHANYUAN ZHANG. Department of Biochemistry and Plant Transformation Core Facility, University of Missouri-Columbia, Columbia, MO 65211. E-mail: zhangzh@missouri.edu Website: www.psu.missouri.edu/muptcf

Soybean functional genomics studies require a routine and consistent transformation system with a desirable recovery frequency using elite public genotypes, especially those that derive EST and BAC data. Procedures using the *bar* gene as a selectable marker have become routine and accessible to the public (Zhang et al., 1999, Plant Cell Tiss Organ Cult 56:37–46; Xing et al., 2000, In Vitro Cell Dev Biol-Plant 36:456–463). However, the utility of these systems has been restricted because many elite public genotypes exhibit low infection with *Agrobacterium tumefaciens*. Very recently, an advance has been made in using the antioxidant L-cysteine to enhance *Agrobacterium* infection of soybean (Ohlroft et al., 2000, Plant Cell Rep, in press). Nonetheless, one effect associated with the use of L-cysteine is its counter-selection, i.e. alleviation of selective pressure by herbicides, limiting consistent recovery of truly transgenic soybean. Therefore, we have sought to optimize both *Agrobacterium* infection and herbicide selection in the presence of L-cysteine. We report here for the first time a consistent transformation of two elite public soybean genotypes, Maverick and Williams 82, previously shown to be recalcitrant to *Agrobacterium* infection (Zhang et al., unpublished). We optimized selection with herbicide glufosinate by evaluating various selection schemes. Transgenic soybean plants derived from these genotypes were routinely developed. Both GUS and leaf-painting assays were conducted on most primary transformants (T_1) and some of their progeny (T_2). Southern blot analysis was conducted on some T_1 plants and a few set of their progeny (T_2) as representatives. Results are analysized and presented.

P-1221

Cold Storage of Micropropagated Bermuda Grass. HAILU M. AYNALEM[1*], Reed Barker[2], and Barbara M. Reed[3]. [1]Horticulture Department, Oregon State University, Corvallis, OR 97331. [2]USDA/ARS National Forage Seed Production Research Center. [3]USDA/ARS National Clonal Germplasm Repository. *E-mail: aynalemh@onid.orst.edu

Genetic resources of vegetatively propagated plants are safeguarded by medium-term storage of micropropagated plants. These procedures provide cost effective backups for field or greenhouse germplasm collections. *Cynodon* (Bermuda grass) germplasm stored as growing plants provides a number of challenges to curators. In-vitro backup collections increase security for existing plant collections. We evaluated a diverse group of *Cynodon* species and selections for 4°C storage including: *Cynodon barberi; C. dactylon vars. dactylon, elegans and polevansii; C. nlemfuensi var. robustus; C. radiatus; C. transvaalensis*. Actively multiplying cultures were transferred to 5-cell tissue-culture bags of MS medium with no growth regulators, 2 bags per accession. Shoots were planted, sealed in bags, grown in the growth room for 1 week (25°C, 16 hr photoperiod, 25 $\mu M \cdot m^{-2} \cdot s^{-1}$), cold acclimatized (8 hr 22°C day/16 hr −1°C night) for 1 week, then stored at 4°C with a 12 hr photoperiod (10 $\mu M \cdot m^{-2} \cdot s^{-1}$). Cold-stored shoots were evaluated at 4 months, then at 2-month intervals by visual and machine analysis. All accessions maintained their green color and produced roots during the first 4-month storage period. From 6 to 12 months some accessions declined significantly while others showed little or no change. Cold-stored shoot cultures can provide backup storage for Bermuda Grass germplasm, however the length of viable storage is highly genotype dependent.

P-1222

Regeneration and Gene Transfer for Herbicide Resistance in Economically Important Bulgarian Alfalfa (*Medicago sativa* L.) Cultivars. A. L. BARBULOVA, A. V. Iantcheva, M. K. Zhiponova, M. G. Vlahova, and A. I. Atanassov. AgroBioInstituite, 2232-Kostinbrod-2, Bulgaria. E-mail: ani_barbulova@agrobioinstitut.org

Alfalfa (*Medicago sativa* L.) is one of the most important forage crops, cultivated on more than 32 million hectares worldwide. Alfalfa is also of considerable worldwide agronomical importance for Bulgaria and occupies presently about 160,000 hectares. The knowledge in the field of tissue culture, transformation techniques and genetic engineering, permits useful traits to be introduced into many diploid and tetraploid *Medicago* species. The processes of somatic embryogenesis and genetic transformation are genotype dependent. To accommodate a genotype that has not been manipulated in culture previously it is necessary either to adapt an established protocol or create a new one, bearing in mind the efficiency imperatives. The objective of the present study was to adopt and optimize the existing procedures and to develop efficient systems for regeneration and transformation for several Bulgarian alfalfa cultivars in order to obtain herbicide resistant plants. Five different Bulgarian cultivars with valuable economic qualities and control line A_{70}/3K were screened for their regeneration capacity. The parameters influencing the development and maturation of somatic embryos were determined. On the base of their superior embryogenic potential cultivar Obnova 10 and control line A_{70}/3K were selected for further transformation experiments. Two systems for *Agrobacterium* mediated genetic transformation were established: leaf disk transformation and vacuum infiltrated seedling transformation. Parameters for high frequency of the gene transfer were optimized. For genetic transformation pCambia 3301 vector, carrying *bar* gene for resistance to contact non – selective herbicide BASTA was used. In vitro putative transformants were obtained and molecular analyses (PCR, Southern blot), confirming the transgenic nature of the plants, were conducted. Treatment of the obtained transgenic plants with herbicide Basta in greenhouse conditions was performed. The efficiency of the two transformation methods was defined.

P-1223

Organ and Tissue Specificity of Expression of a Reporter Gene Driven by Various Promoters in Transgenic Turfgrass. CHHANDAK BASU[1]*, Debra C. George*, Albert P. Kausch*, and Joel M. Chandlee*. *Dept. of Plant Sciences, University of Rhode Island, Kingston, RI 02881. [1]E-mail: chhandak@yahoo.com

Use of biotechnology for the purpose of plant trait enhancement and modification is a powerful tool available to plant breeders. The objective of this project to evaluate and assess some novel promoters useful for turfgrass transformation. We have previously optimized different parameters for gene transfer to turfgrass by the biolistics approach. Here we report the transient expression of a reporter gene *uidA* (GUS) driven by a variety of different promoters in different organs and tissues in turfgrass (cv. Penn A4). Leaves and roots were excised from the plants on the day of the bombardment and arranged on petri dishes. Embryogenic calli from turfgrass seeds were generated and two-month old calli were selected for bombardment. We tested turfgrass calli, roots and leaves (young and mature) with four different promoters (ubiquitin rice, ubiquitin corn, ubiquitin-3 potato and CaMV 35S), driving expression of *uidA* (GUS) as a reporter gene. A histochemical assay for GUS was performed after 48 hours of bombardment. The relative efficiency of each promoter was the same in all these three tissue types. We determined that the monocot specific promoters, ubiquitin rice, expressed the highest number of GUS hits in calli, leaf and root tissues followed by ubiquitin corn. The other two dicot specific promoters (ubiquitin-3 potato and CaMV 35S) did not perform very well, as expected. Finally we observed that the relative efficiency of all promoters with respect to GUS expression was dramatically higher in mature leaves compared to young leaves.

P-1224

Genetic Manipulation of Lignin Biosynthesis in Tall Fescue. LEI CHEN and Zengyu Wang. Forage Biotechnology Group, Samuel Roberts Noble Foundation, 2510 Sam Noble Parkway, Ardmore, OK 73401. Email: clei@noble.org

Lignification of plant cell walls is the major factor responsible for lowering digestibility of forage tissue and that such changes in dry matter digestibility greatly contribute to the lowering of nutritive value of forage grasses. Tall fescue (*Festuca arundinacea* Schreb) is the predominant cool-season forage species in North America. Anatomical comparisons of tall fescue stems at six different developmental stages revealed a gradient increase in lignification with progressive maturity. Klason lignin content, S lignin content, and S/G ratio increased with progressive maturity of tall fescue stems. The in-rumen digestibility is negatively correlated with lignin content, S lignin content, as well as S/G ratio. Caffeic acid O-methyltransferase (COMT) and cinnamyl alcohol dehydrogenase (CAD) are key enzymes involved in lignin biosynthesis. cDNAs of COMT and CAD were cloned from tall fescue. Transgenic tall fescue plants were generated using gene constructs harboring COMT and CAD cDNA sequences by using microprojectile bombardment. Strong down-regulation of CAD resulted in decreased total lignin content, and reduction in S lignin and H lignin.

P-1225

Establishment of a *Brachypodium distachyon* Transformation System. P. CHRISTIANSEN(1), M. Folling(2), I. Lenk(2), Y. Levy(1), and K. K. Nielsen(2). (1)Risø National Laboratory, Plant Research Department, Frederiksborgvej 399, DK-4000 Roskilde, Denmark and (2)DLF-Trifolium, Danish Plant Breeding, Højerupvej 31, DK-4660 Storre Heddinge, Denmark. E-mail: pernille.christiansen@risoe.dk

In the last few years *Brachypodium distachyon* (Pooidea) has been investigated and evaluated as a monocot model plant for grass biotechnology. The genome size of *B. distachyon* is comparable to *Arabidopsis thaliana*, and ecotypes having the ploidy 2N, 4N or 6N are available. The seed-to-seed life cycle is 15 weeks for the fast growing ecotypes and 25 weeks for 2N plants due to the need of vernalization. Preliminary studies showed that *B. distachyon* is fast and easy to handle in tissue culture, e.g. only 0–5% albinos regenerated. Transient transformation of 2N and 4N ecotypes was established using microprojectile bombardment of embryogenic callus produced from immature embryos. *Bar* and *uidA* (conferring resistance to the herbicide bialaphos, and encodeing the GUS reporter enzyme, respectively) were used for the transformation experiments, and up to 6000 blue GUS-positive spots per bombardment were obtained. Stable transgenic plants were selected on bialaphos, and the plants were transferred to soil 15–17 weeks after initiation of callus production. The described characteristics make *Brachypodium distachyon* very qualified as model plant for functional genomics in grasses.

P-1226

Switchgrass Anther and Microspore Culture. J. K. MCDANIEL, Z. Tomaszewski, and B. V. Conger. Dept. of Plant Sci. and Landscape Sys., University of Tennessee, Knoxville, TN 37996. E-mail congerbv@utk.edu

In recent months we have expended considerable effort toward attempts to obtain haploid plants of switchgrass (Panicum virgatum L.). Success with anther culture techniques has been limited due to the low frequency of responding anthers and the long period between initial plating and obtaining the first response (callus and/or embryo-like structures). Additionally, most of the obtained calluses were nonembryogenic and did not regenerate plantlets. From a few embryogenic calluses it has been possible to regenerate a large number of green plants. However, successful plant establishment may take as long as 16 months because of poor rooting. Another problem has been lack of satisfactory growth and development of regenerated plantlets in greenhouse conditions. After transferring plantlets from in vitro culture into soil, growth is slow. Occasionally, the plants turn yellow and die in spite of proper and careful maintenance. Because of low efficiency of anther culture, we are currently investigating liquid culture of isolated microspores. This method includes isolation of microspores from spikelets, their purification and culture in liquid media. To date we have obtained only cell divisions and multicellular structures.

P-1227

Stable Transformation of Bahiagrass (*Paspalum notatum*) by Particle Bombardment. T. Gondo[1], RYO AKASHI[1], O. Kawamura[1], and Franz Hoffmann[2]. [1]Faculty of Agriculture, Miyazaki University, Miyazaki 889–2192, Japan. [2]Department of Developmental and Cell Biology, University of California, Irvine, CA 92697–2300. E-mail: rakashi@cc.miyazaki-u.ac.jp

We have improved our previously published technique for plant regeneration from seed-derived embryogenic callus of bahiagrass (Akashi et al., 1993) by keeping the callus size small through regular partition. This modified culture remains regenerable for at least 18 months and results in a decrease of albino plants among regenerants. Also, in transformation experiments, an increased surface area is available for gene delivery, and a greater number of cells can potentially regenerate. Using a simple self-built particle inflow gun with improved technical features, we have optimized delivery conditions and bombarded the small calli with pDB1 (Becker et al., 1994), a construct carrying both the GUS reporter gene and a bialaphos resistance gene (*bar*). Numerous calli have been selected on bialaphos-supplemented medium and subjected to regeneration conditions. These calli remained resistant and continue to display GUS activity (Gondo et al., 2000). Shoots as well as embryo-like structures formed from resistant calli under regeneration conditions and green plants were obtained. Akashi et al., (1993) Plant Sci. 90: 73–80; Becker et al., (1994) Plant J. 5: 299–307; Gondo et al., (2000) Proceeding of Molecular Breeding of Forage Crops 2000: p. 123

P-1228

Development of Roundup Ready Creeping Bentgrass. LISA LEE[1], Shirley Guo[2], Wayne Horman[1], Eric Nelson[1] and Robert W. Harriman[1]. [1]Turfgrass Variety Development and Biotechnology Department, The Scotts Company, 14111 Scottslawn Road, Marysville, OH 43041 and [2]Monsanto, 700 Chesterfield Parkway North, St. Louis, MO 63198. E-mail: lisa.lee@scottsco.com

Creeping bentgrass is a cool season grass that is used mainly on golf course putting greens, tees, and fairways. Currently, golf course superintendents can not effectively control serious grass weeds on golf courses such as *Poa annua* and *Poa trivialis*. This often leads to management of both the desirable and undesirable grasses in an effort to avoid turf thinning from disease, insect or temperature stress, which thereby increases input needs such as fungicide, insecticide, water and fertilizer. Herbicide tolerant creeping bentgrass can provide selective control of weeds, and thereby reducing turf management needs of spraying pesticides through the elimination of weeds. In addition to reduced inputs, improved turf management can result in higher uniformity and quality of turf. Through particle bombardment transformation, herbicide resistance to Roundup(r) has been introduced into creeping bentgrass. We used embryogenic callus cultures derived from mature seeds for bombardment. Transgenic plants regenerated from glyphosate resistant callus cultures have displayed high levels of herbicide tolerance in both greenhouse and field herbicide spray tests. Roundup Ready(r) creeping bentgrass is in its final stages of product development. As with all previous products developed with modern biotechnology, RRCB must pass a thorough review by the Environmental Protection Agency and the U.S. Department of Agriculture prior to commercialization. Highlights of RRCB product development including transformation and regulatory studies will be presented.

P-1229

Isolation and Characterisation of Three Cinnamyl Alcohol Dehydrogenase Homologue cDNAs from Perennial Ryegrass. DAMIAN LYNCH, Angela Lidgett, and German Spangenberg. Plant Biotechnology Centre, Agriculture Victoria, Department of Natural Resources and Environment, La Trobe University, and CRC for Molecular Plant Breeding, Bundoora, Victoria 3086, Australia. Email: damian.lynch@nre.vic.gov.au

Cinnamyl Alcohol Dehydrogenase (CAD) governs the last comitted step of the monolignol biosynthetic pathway, making it an ideal target for manipulating lignin biosynthesis. Three homologue cDNAs were isolated from a *Lolium perenne* seedling cDNA library. Expression analysis, sequence and phylogenetic comparisons disclose the divergent nature of these three CADs. LpCAD3 is a typical CAD gene closely related to maize and sugarcane CADs involved in lignification while LpCAD1 and LpCAD2 are divergent defence-related CADs. Transcript abundance of the three CADs varied between plant organs and developmental stages but all were similarly upregulated in response to a mechanical wounding stimulus. Sense and antisense vectors have been constructed for the down-regulation and over-expression of CAD in transgenic plants. The complete gene sequence of LpCAD2 has been solved to allow for analysis of gene regulatory sequences. The project allows for modification of the lignin profile of pasture grasses to improve digestibility by down-regulating, through antisense and sense suppression in transgenic plants, the expression of genes encoding a key enzyme involved in the biosynthesis of monolignols.

P-1230

Herbicide Resistant White Clover (*Trifolium repens* L.) Regenerated from Seedling Cotyledons. J. L. NASH, S. S. Croughan, B. C. Venuto, and B. S. McClain. LSU Agric. Ctr., Rice Research Station, P.O. Box 1429, Crowley, LA 70527, and LSU Agric. Ctr., Southeast Research Station, Franklinton, LA 70438. E-mail: jnash@agctr.lsu.edu

The white clover cultivar LA-S1 was developed in Louisiana and is well adapted to the climate of the southern United States. However, the susceptibility of this cultivar to the major broad-leaf herbicides used on pasture fields has limited its use as a forage crop in mixed species swards. The goal of this study was to develop an herbicide resistant variety of LA-S1 white clover for use by farmers and ranchers in Louisiana and areas with a similar climate. Seedling cotyledons were used to mass-produce shoots via organogenesis. These shoots were then screened by plating on a selection medium containing 0.0, 0.5, 1.0, 5.0 or 10.0-PPM Cadre (R) herbicide. All shoots that produced roots were potted and transferred to the greenhouse where they were sprayed with 0.25 lb ai/A Cadre(R) herbicide to test for resistance. The surviving regenerants will be evaluated in field trials for agronomic charactersitics. Selections from these trials will be intercrossed to produce Syn 1 seed for further evaluation and development.

P-1231

Investigation of Biolistics as an Alternative Transformation Method for Narrow-leafed Lupin (*Lupinus angustifolius*). K. RATANASANOBON, S. Wylie, and M. G. K. Jones. W. A. State Agricultural Biotechnology Centre, Murdoch University, Murdoch, WA, Australia, 6150. E-mail: ratana@central.murdoch.edu.au

The potential for lupins as an agricultural crop has been recognized worldwide. Despite work going on in several different laboratories around the world, the efficiency of stable gene transfer by *Agrobacterium* remains at approximately 1% for modern varieties. Here we investigate particle bombardment as an alternative method of gene transfer. Preliminary studies were carried out by using tungsten particles (~1μm in diameter) coated carrying DNA encoding the *gus* reporter gene and the *bar* gene for selection of transformants. Particles were accelerated into embryonic axes by helium inflow. A number of parameters were studied including plasmid preparation methods, pre-treatment of target tissue, particle density, helium pressure, DNA concentration, and the distance between the injector and target tissue (target distance). Expression as determined by blue spots of GUS staining has been increased significantly, but stable transformation, as determined by survival of shoots in selection medium (containing 10 m g/ml PPT), has not been achieved. Analysis of GUS expression seven days post-bombardment, and penetration depth of the tungsten particles in target tissue have suggested that the particles did not penetrate to the LII and LIII tissue layers of embryogenic axes, which give rise to organs including inflorescence of grown plants.

P-1232

In Vitro Preservation of Bermuda Grass Germplasm. BARBARA M. REED[1*], Nan Wang[2], Jeff D'Achino[1], and Reed E. Barker[3]. [1]USDA/ARS National Clonal Germplasm Repository, 33447 Peoria Road, Corvallis, OR 97333; [2]Horticulture Dept., Oregon State University; and [3]USDA/ARS National Forage Seed Production Research Center. E-mail: reedbm@bcc.orst.edu

Genetic conservation of vegetatively propagated grasses requires constant care of pot cultures or carefully separated field plots. Medium and long-term storage procedures to safeguard this germplasm would be more cost effective and provide a backup for field or greenhouse germplasm collections. Cynodon (Bermuda grass) germplasm is mostly stored as growing plants. We evaluated a diverse group of Cynodon species and selections for storage in liquid nitrogen at −196°C including: *Cynodon barberi; C. dactylon vars. afghanicus, dactylon, aridus, coursii, elegans and polevansii; C. nlemfuensi vars. nlemfuensis, robustus; C. incompletus var. incompletus; C. aethiopicus; C. arcuatus; C. incompletus var. hirsutus; C. plectostachyus; C. radiatus; C. transvaalensis; C. dactlyon x C. transvaalensis; C. dactylon x C. nlenfuensis*. In-vitro grown shoots were cold acclimated for 4 weeks and 0.8 mm shoot tips were encapsulated in alginate beads, pretreated in sucrose solutions, dried under laminar flow for 6 h and immersed in liquid nitrogen for at least 1 h. Regrowth of cryopreserved shoot tips ranged from 20% to 90%. Seventeen of the 23 accessions (74%) had greater than 40% regrowth. The remaining accessions had 20–38% regrowth. Fifty encapsulated meristems of each of 34 Cynodon accessions were stored and are now held as a base collection in liquid nitrogen at the National Center for Germplasm Resources Preservation (NCGRP) in Fort Collins, Colorado.

P-1233

Microprojectile Bombardment Transformation of Perennial Ryegrass (*Lolium perenne*) for Manipulation of Flowering Behaviour. Kim Richardson, Kerry Templeton, Igor Kardailsky, Bruce Veit, and Greg Bryan. Plant Breeding and Genomics, AgResearch, Grasslands Research Centre, Private Bag 11008, Palmerston North, New Zealand. E-mail: kim.richardson@agresearch.co.nz

In New Zealand, perennial ryegrass (*Lolium perenne* L.) is the predominant component in nearly all pasture mixtures, with perennial ryegrass and white clover forming the basis of permanent pastures for dairy production, sheep, and cattle. However a decline of feed value in perennial ryegrasses is associated with the onset of flowering. This has a negative impact on the agronomic value as photosynthate is diverted away from leaves into developing inflorescences. We are interested in manipulating the expression of flowering genes in an attempt to develop non-flowering cultivars. To this end we have established a transformation system for perennial ryegrass using a microprojectile bombardment strategy. Transgenic plants expressing GUS and GFP have been obtained for two elite New Zealand cultivars. Molecular analysis of these plants along with preliminary data for plants transformed with genetic constructs expressing an arabidopsis *FT* and a *TFL*-like gene will be presented.

P-1234

Isolation and Characterisation of a Fructosyltransferase Homologue cDNA from Perennial Ryegrass. KATHERINE TERDICH, Xenie Johnson, Angela Lidgett, German Spangenberg. Plant Biotechnology Centre, Agriculture Victoria, Department of Natural Resources and Environment, La Trobe University and CRC for Molecular Plant Breeding, Bundoora, Victoria 3086, Australia. E-mail: katherine.terdich@nre.vic.gov.au

Fructans are polyfructose molecules that consist of linear or branched fructose chains attached to a sucrose precursor. They are the major storage carbohydrate of approximately 15% of angiosperm species. Fructans play an important physiological role in carbohydrate partitioning by buffering temporary imbalances between photosynthetic carbon supplies and demands for growth and development. Temperate grasses, such as ryegrasses and fescues, accumulate high amounts of fructans in response to environmental stresses such as drought and cold and play an important physiological role in photosynthate. This project aims to manipulate fructan biosynthesis for the production of pasture grasses with enhanced persistence, tolerance to abiotic stress and quality. One full length cDNA was isolated from a *Lolium perenne* seedling cDNA library and sequence analysis revealed a 1944 bp open reading frame. This gene (LpFT1) encoded a putative fructosyltransferase protein of 648 aa. LpFT1 had high amino acid homology to the fructosyltransferase from Barley (Hv6SFT) involved in fructan biosynthesis. LpFT1 was found to be a single copy gene in ryegrass and to be constitutively expressed. Transcript levels were found to show highest expression in roots and shoots at early seedling growth and in mature leaf sheath. A set of transgenic tobacco plants have been generated containing the ryegrass 6SFT homologue, LpFT1 and also the barley 6SFT. To identify transgenic plants, PCR screening was undertaken, independent transgenic plants were found to contain both the selectable marker and the transgene. The mitotically stable integration of the transgene into the genome of selected PCR clones was demonstrated by Southern hybridization analysis. Transgenic tobacco plants were found to contain between 1–5 integrated copies of the transgene. The expression of the introduced transgene in transgenic tobacco plants was demonstrated by northern hybridisation analysis. Biochemical analysis and performance under drought conditions of the transgenic plants is currently being assessed. A set of transgenic ryegrass plants has been created and molecular analysis is being undertaken.

P-1235

Encapsulation of Seedling-like Somatic Plantlets for Producing Smooth Cordgrass (*Spartina alterniflora*) Synthetic Seeds with High Seedling Establishment Rates. HERRY S. UTOMO, Ida Wenefrida, and Timothy P. Croughan. Rice Research Station, Louisiana State University Agricultural Center, 1373 Caffey Rd., Rayne, LA 70578. E-mail: hutomo@agctr.lsu.edu

A high seedling establishment rate is critical for the application of synthetic seeds. This study was conducted to 1) evaluate the capability of *S. alterniflora* somatic embryos (SEs) and seedling-like somatic plantlets (SLSPs) 3, 4, and 5 mm in length to produce synthetic seeds with high seedling establishment rates and 2) determine conditions required to store the resulting synthetic seeds. SEs were produced from proembryogenic masses on semi-solid regeneration medium. SLSPs were micro-plantlets obtained from germinating mature SEs. These SEs and SLSPs were encapsulated in 2% (w/v) calsium alginate to produce synthetic seeds. Average seedling establishment rates of synthetic seeds derived from SE, SLSP3, SLSP4, and SLSP5 were respectively 5, 71, 83, and 86%. Synthetic seeds derived from SLSPs had about a two-week maturity advantage over SE-derived synthetic seeds. Further evaluation on SLSPs indicated that SLSP5 was fully autotropic. Synthetic seeds derived from this materials, therefore, will have better chance to survive under natural conditions compared to that derived from other SLSPs or SE. *S. alterniflora* synthetic seeds could be stored at 1 to 5°C inside clear closed containers with low light intensity of 5 m $E{\cdot}m^{-2}{\cdot}s^{-1}$ for 6 weeks without significant loss of viability. Pre-treatments using ABA or desiccation could not further prolong the storage period.

P-1236

Genetic Diversity of Louisiana Smooth Cordgrass (*Spartina alterniflora*) Based on Random Amplified Polymorphic DNA Analysis. IDA WENEFRIDA, Herry S. Utomo, and Timothy P. Croughan. Rice Research Station, Louisiana State University Agricultural Center, 1373 Caffey Rd., Rayne, LA 70578. E-mail: iwenefrida@agctr.lsu.edu

Genetic diversity has been a critical and debated issue related to the use of plants for coastal erosion control and wetlands reclamation. The objective of this study was to determine the genetic diversity of *S. alterniflora* using random amplified polymorphic DNA (RAPD) analysis. A total of 95 *S. alterniflora* accessions collected along Louisiana coastal marshes were used in this study. Twelve decamers were employed to generate DNA fragments for each accession. A total of 225 fragments were obtained with the 12 primers, and among these, 136 fragments (60%) were polymorphic. The size of fragments ranged from approximately 180 to 2,300 base pairs. The proportion of *S. alterniflora* accessions with polymorphic RAPD fragments ranged from 1(rare) to 88% (common) with a mean frequency of 40%. A total of 4,465 pairwise comparisons based on 136 polymorphic RAPD markers were made. Cluster analysis revealed that the 95 *S. alterniflora* accessions used in this study felt into three genetic groups, each of which is separated by genetic distance of more than 0.90. Plotting the members of each group according to their origins indicated that each group occupied a large area and was well-dispersed across coastal region. There was no apparent specific genetic pattern associated with geographical location. The mean genetic diversity index was 0.62. The overall data suggested that Louisiana smooth cordgrass exhibited a large amount of genetic diversity.

P-1237

Agrobacterium-mediated Co-transformation of Dessert Banana (Musa AAA cv. Grand nain): Strains, Efficiency, Copy Number and Insert Integrity. N. L. ADAMS, K. Summers, H. Percy, G. Nisbet. Syngenta, Jealott's Hill International Research Centre, Bracknell, Berkshire, RG42 6EY, United Kingdom. E-mail: nicola.adams@syngenta.com

To facilitate the stacking of multiple genes and multiple transgenic traits into the commercial dessert banana cultivar Grand nain (which is a sterile, parthenocarpic triploid in the Cavendish subgroup), an *Agrobacterium*-mediated co-transformation system for embryogenic cell suspensions was developed. Overall transformation efficiencies, insert copy number and insert integrity were strain dependent, and co-transformation efficiencies were determined by a combination of the Agrobacterium strain used and their relative ratios. Co-transformation frequencies of over 50% were achieved, as identified by molecular and phenotypic analysis of primary transformants.

P-1238

Functional Properties of Transgenic Rye (*Secale cereale* L.) Stably Expressing High Molecular Weight Glutenin Subunits from Wheat (*Triticum aestivum* L.). F. ALTPETER[1]*, J. C. Popelka[2], H. Wieser[3], R. Kieffer[3]. [1]University of Florida, Agronomy Department, Laboratory of Molecular Plant Physiology, 2191 McCarty Hall, P.O. Box 110300, Gainesville FL 32611–0300; [2]Institut für Pflanzengenetik und Kulturpfanzenforschung Gatersleben, AG Gentransfer, Corrensstrasse 3, 06466 Gatersleben, Germany; and [3]Deutsche Forschungsanstalt fuer Lebensmittelchemie (DFA), AG Struktur/Wirkungsbeziehungen bei Biopolymeren *Email: faltpeter@mail.ifas.ufl.edu

The production of transgenic rye (*Secale cereale* L.) plants stably expressing different high molecular weight glutenin subunits (HMW-GS) from wheat is described. Identification and combination of interactive factors allowing gene transfer and recovery of transgenic events without compromising on the regeneration potential were important factors in developing a routine transformation protocol for rye. Different selection strategies using the selectable markergenes *bar* or *npt*II resulted in similar transformation efficiencies in the range of 2 to 4% of the cultured explants. A low transgene copy number was observed in most of the transgenic plants and 40% of the transgenic plants had a single copy insert. Functional properties and bread making quality of dough from homozygous rye seeds stably expressing one or two HMW-GS will be discussed. Expression cassette of HMW-gs 1Ax1 was kindly provided by Peter Shewry (IACR-Long Ashton, Bristol, UK) and of 1Dx5 and 1Dy10 by Anne Blechl (ARS, USDA, Albany, CA, USA). This work was supported by the Lochow—Petkus GmbH in Bergen (Germany) together with the German Bundesministerium für Bildung, Wissenschaft, Forschung und Technologie (BMBF).

P-1239

Characterization of Carotenoid Cleavage Enzymes. MICHELE E. AULDRIDGE and Harry Klee. Plant Molecular and Cellular Biology, University of Florida, Gainesville, FL 32611. E-mail: mauldrid@ufl.edu

Carotenoids are C_{40} compounds, consisting of 8 isoprene units and a series of conjugated double bonds. Apocarotenoids are derived from the breakdown of carotenoids, which frequently involves oxidative cleavage at carotenoid ends. Several volatile apocarotenoids have been shown to be flavor and aroma compounds detectable by humans at very low levels and many act as insect attractants. Other apocarotenoids are biologically active, such as abscisic acid (ABA), a plant hormone involved in the regulation of growth, seed dormancy, and adaptation to osmotic stress. A family of nine genes similar to the maize *Vp14* gene, encoding a dioxygenase responsible for ABA synthesis, has been identified in the Arabidopsis genome. Four of the proteins encoded by these genes show highest homology with *Vp14* and are termed 9-cis-epoxy-dioxygenases (NCEDs). Due to their divergence from the rest of the family members, the remaining five are less likely to be involved in ABA biosynthesis and are termed Related to Carotenoid Dioxygenases (RCDs). My project entails the characterization of three members of the RCD gene family, RCD1, 7 and 8. *In vitro* assays have confirmed the identification of RCD1 as a carotenoid dioxygenase by demonstrating its ability to symmetrically cleave a variety of carotenoids at their 9–10 and 9'-10' double bonds. It is likely that identification and characterization of carotenoid cleavage enzymes will lead to a greater understanding of flavor development in fruits as well as the biological significance of apocarotenoids.

P-1240

In Vitro Conservation of Ribes Species. *ERICA E. BENSON, *Graham Sherlock, **Dominique Dumet, ##Barbara M. Reed, ##Jeanine M. DeNoma, #William Block, #Roger Worland, *Harry Staines. *Plant Conservation Group, School of Science and Engineering, University of Abertay Dundee, Bell Street, Dundee, DD1 1HG, Scotland. E-mail: e.e.benson@abertay.c.uk, reedbm@bcc.orst.edu

Conserving vegetatively propagated crops is problematic as seed storage is not an option. Cryopreservation of *in vitro* shoot-tips provides a complementary approach to field genebanks. The USDA-ARS, NCGR, Oregon, holds cryopreserved collections of *Ribes* and a pilot genebank is established at the University of Abertay Dundee, Scotland. These organizations aim to develop *in vitro* conservation protocols that can be reproducibly applied in different international genebanks. The effects of pre-treatments using cold hardening and sucrose pregrowth have been assessed; both enhance post-cryopreservation survival. Responses of different genotypes to a range of cryopreservation protocols have been determined in USA and Scottish collections. Differences in survival can arise with respect to method used and operational variations between laboratories. Differential Scanning Calorimetry (DSC) has been applied to ascertain the basis of different responses to encapsulation/dehydration techniques and will assist the application of cryopreservation to recalcitrant genotypes. To aid cryopreservation methods development a US-European Collaborative Project has recently been launched. The aim being to coordinate technology transfer by evaluating critical point factors in storage protocols. As a result, robust storage procedures will become routinely applicable in geographically dispersed genetic resources centers.

P-1241

Morphological and Photosynthetic Status of Micropropagated Strawberries to Mycorrhization. BOZENNA BORKOWSKA. Department of Plant Physiology and Biochemistry, Research Institute of Pomology & Floriculture, 96–100 Skierniewice, Poland. E- mail: bborkow@insad.pl

In natural systems, plants are usually colonised by symbiotic fungi. The plants produced *in vitro* are free of any microflora. During the last years, the mycorrhizal technology has been used in a number of micropropagated horticultural crops in order to improve their growth as well as to enable host plants to tolerate or withstand the impairing effects of abiotic and biotic stresses. We attempted to study the effect of colonisation by arbuscular mycorrhizal fungi (AMF) of micropropagated strawberry plantlets on their morphology and photosynthetic activity (measured by chlorophyll fluorescence method). The microshoots obtained during standard multiplication *in vitro* were rooted either *in vitro* (traditionally) or *ex vitro*. Inoculum (produced by BIORIZE, France) was applied to not-rooted shoots or to previously rooted (*in vitro*) ones, during their acclimatisation in a growth chamber. When shoots were rooted *ex vitro*, simultaneously with root formation they were colonised by fungi and at the same time the plantlets passed from heterotrophic to autotrophic metabolism. The overall plant size and within-plant relationships between different organs (such as root-to-shoot ratios), photosyntetic activity and water status, were significantly better for AMF-plants than for plants without symbionts. When inoculation was carried out with not acclimatised but previously rooted shoots (*in vitro*) – the reverse may occur. In this last model of mycorrhization, the roots are colonised by fungi long before developing photosynthetic activity and as a result they might be more parasitic than symbiotic.

P-1242

Pineapple Genomics: Isolation and Characterization of Tissue-enhanced cDNAs and Promoters from Cultivated Pineapple. Leon W. Neuteboom and DAVID A. CHRISTOPHER. Department of Molecular Biosciences & Bioengineering, University of Hawaii, 1955 East-West Rd. Honolulu, HI 96822. E-mail: dchr@hawaii.edu

The purpose of this project is to increase the permanent genetic resources for the improvement of cultivated pineapple (*Ananas comosus*). Protocols were developed to isolate high-quality mRNA from a variety of pineapple tissues and to construct cDNA libraries. A reverse northern approach was utilized to differentially screen a root cDNA library using tissue-specific radiolabeled first-strand cDNA probes derived from poly(A)+ mRNA of root, fruit and aerial tissues. Clones were categorized into 14 classes based on their different tissue-based expression characteristics. 40% of the clones were classified as constitutive, being expressed about equally in all three tissues. Approximately 25% of the clones were expressed higher in roots than the other tissues. 32 unique clones were sequenced and preliminarily identified based on sequence homology. Six of the clones selected contained coding regions not significantly homologous to any accessions derived from other organisms (*Arabidopsis*, rice, or cyanobacteria). The expression of the 26 remaining clones was analyzed in detail in 14 different tissues of a mature plant, as well as in roots and aerial tissue from 3, 6, 13, 20 month-old developing plants and during 5 stages of fruit development. Six cDNAs were identified as expressed preferentially in roots with no expression in fruits. A root-specific promoter for one of these genes was isolated by screening a genomic library. The resources developed in this work (tissue-regulated promoters, gene probes and DNA sequence information) will facilitate physical mapping, marker-assisted breeding and genetic engineering efforts for pineapple improvement.

P-1243

Multiple Transgene in Grape for Seedless and Stress Tolerance. V. COLOVA-TSOLOVA[1], J. Lu[1], I. Tsvetkov[2], A. Atanassov[2], and Avi Perl[3]. [1]Center for Viticulture Science & Small Fruit Research, Florida A&M University, 6505 Mahan Drive, Tallahassee, FL 32317, [2]A. Atanassov, National AgroBio Institute, 2232 Kostinbrod- 2, Bulgaria, and [3]Dept. Fruit Tree Breeding & Molecular Genetics,ARO Volcani Center, P.O. Box 50250, Bet-Dagan, Israel. E-mail: Violetka.Colova@famu.edu

The efficiency of a genetic transformation procedure depends on several factors: the right choice of the plasmid construct, optimal conditions for transformation, skill to regenerate plants from transformed tissue, proper and accurate selection and possibility of developing intact transgenic plants.The crucial requirement in the process of transformation is the availability of cells having both the competency to be transformed and that to regenerate into plants subsequently. Thus, embryogenic cells in suspension are very promising candidates for successful gene transfer in grape. The present work was aiming development of highly efficient technology for gene transfer in grape: enable to perform multiple transformation relevant not only for direct genetic improvement, but also to be competent tool for basic study of the gene expression in grape. Two protocols for initiation Somatic Emriogenesis (SE) respectively from in vivo anthers and in vitro young leaves were developed for important commercial grape cultivars and rootstocks. Additional protocol for SE using as a primary explants, in vivo petioles and anthers was established for Muscadine grape (*Vitis rotundifolia*) cv. Fry. Emryogenic lines in suspension culture have been successfully inoculated and maintained:*V. vinifera* cvs- Velika, Merlot, Chardonnay, Flame Seedless; rootstock cvs- 110 Richter, Rupestris du Lot. Well working approach for synchronizing and monitoring of each developmental state of SE has been used to ensure extremely high frequency of the transformation events. As a result, high efficiency and uniform regeneration of the putative transgenic plants is reported. Very efficient genetic selection (selective agent equal to 100mg/l kanamycin were performed in embryogenic cell suspension cultures, following the *Agrobacterium* –mediated gene transformation of PMCs (Proembryogenic Mass of Cells) as a target tissue for alien gene delivery. Histochemical *Gus* assay and molecular analysis including *PSR* and *Southern blot* were used to confirm the multiple 'transgene.' The traits of interest are seedless, disease (viral, bacterial, fungal) and cold tolerance. Some of the results from evaluation of the transgenic lines expressing various gene of interest are presented.

P-1244

In Vitro Culture of Cranberry (*Vaccinium macrocarpon* Ait.): Effect of Zeatin and 2ip On Shoot Proliferation. SAMIR C. DEBNATH. Agriculture and Agri-Food Canada, Atlantic Cool Climate Crop Research Centre, P.O. Box 39088, 308 Brookfield Road, St. John's, Newfoundland A1E 5Y7, Canada. Email: debnaths@em.agr.ca

Shoots of three cranberry (*Vaccinium macrocarpon* Ait.) clones from natural stands in Newfoundland, were initiated *in vitro* from nodal explants on a nutrient medium containing the plant growth regulators, N^6-[2-isopentenyl]adenine (2iP) (12.3 FM) or zeatin (5.7 FM). The effect of zeatin was compared with that of 2iP in establishment and proliferation phases. Zeatin was more effective than 2iP, and induced proliferation of about 2 to 3 times as many shoots over two culture periods. Best total shoot proliferation was obtained when nodal segments were cultured in the medium supplemented with 2–4 FM zeatin. Shoots rooted well *in vitro* in the same medium used for shoot proliferation, but without any growth regulators.

P-1245

The Importance of Peptide Transporters for the Translocation of Nitrogen in *Arabidopsis thaliana*. DANIELA DIETRICH and Doris Rentsch. Institute for Plant Sciences, Plant Molecular Physiology, University of Berne, Altenbergrain 21, CH-3013 Berne. e-mail: daniela.dietrich@ips.unibe.ch

For quite a long time there has been good evidence for the transport of small peptides (two to three amino acids long) in plants. As early as the late seventies physiological experiments have shown that during the germination of barley a peptide transport system is active. Nonetheless not much is known about the significance of peptide transporters regarding total nitrogen uptake or distribution in whole plants. Transport of peptides would be an efficient way for nitrogen translocation in situations where massive protein hydrolysis and rapid relocation of the hydrolysis products takes place. This could either be during senescence, when leaf proteins are degraded and used for seed loading or during germination, when protein storage bodies are mobilized to sustain seedling growth. Analysing peptide transporters from Arabidopsis thaliana, we could show so far that *AtNTR1* (nitrogen transporter) has a K_m of 20 microM for the transport of the dipeptide Leu-Leu. Competition studies with various peptides showed that transport activity does not depend on amino acid composition, but rather on peptide length, with transport being limited to peptides of two or three amino acids. These results fit well with an implied role in the transport of products of protein hydrolysis. Analysis of seeds from transgenic plants expressing the *uidA* gene under the control of the *AtNTR1* promoter revealed expression in a layer directly underneath the testa as well as in the embryo proper. This suggests an involvement of *AtNTR1* in seed development and/or germination. Data on the analysis of T-DNA insertion lines and the more detailed characterization of a new second peptide transporter, *AtNTR2*, will be presented to demonstrate the significance of peptide transporters for nitrogen distribution in plants.

P-1246

Molecular Breeding of Fruit Crops. SERGEY V. DOLGOV, Vadim G. Lebedev, and Sergey A. Taran. Branch of Shemyakin and Ovchinnikov Institute of Bioorganic Chemistry, RAS, 142290 Puschino, Moscow Region, Russia. E-mail dolgov@fibkh. serpukhov.su

The economic importance of horticultural crops has led to selection and breeding over thousands of years. This practice has resulted in relatively few genotypes and, therefore, in a restricted germplasm base. Recent developments in biotechnology have provided for an alternate approach to horticultural crops improvement through the introduction of genes encoding desirable traits. Most research to date has focused on genes conferring resistance to viruses, bacteria, insects and fungi. However, attention has also been given to genes that regulate such parameters as freezing tolerance or fruit taste. Based on developed transformation protocols the gene of supersweet protein thaumatin II was transferred to apple (Melba) and pear (Burakovka) varieties. Its introduction into plant genomes confirmed by PCR analysis and expression by Western blotting. Some of transgenic lines demonstrated sweet taste in the leaves of greenhouse plants. Herbicide resistant fruit rootstock is a new way conferring selectivity and enhancing fruit crop safety and production. The bar gene cloned in "Bioengineering Center" RAS have been used in our research for obtaining phosphinotricine-resistant apple rootstocks. This gene encodes a phosphinotricine acetyl transferase (PAT) which convert PPT into the non-toxic acetylated form and prevent the toxicity accumulation in plant tissues. Based on development transformation protocols more than 20 transgenic plant of clonal apple rootstock N545 and pear GP217 were obtained. The bar integration into plant genomes was confirmed by PCR analysis. The transgenic plants expressing various levels of PAT were resistant for commercial herbicide "Basta". For phytopathogenes resistance improvement the small antibiotic-like protein (defensins) gene cloned from Rafanus sativus have been transferred to apple and pear varieties. PD gene introduction in genomes has been confirmed by PCR analysis and its expression is detectable by Western blot testing. In contrast to antimicrobial peptides having non-plant origin (cecropin, magainin, tachyplesin and others) PDs are evidently optimal for expression in plants. It is essential that PDs have not activity against cells of higher eucariots. For studying the possibility of frost resistance improvement, the sour cherry transgenic plants with the gene of winter flounder antifreeze protein (AFP) have been obtained. The problems of efficient foreign gene transfer and expression as its commercial application for fruit trees breeding will be discussed. In 2000 year we have started field tests of transgenic apple, pear, strawberry and chrysanthemum in certified field plots of All-Russia Institute of Fruit Breeding (Orlovskaya obl.). More than five hundreds of transgenic plants have been planted to undergone studies of the field expression of transferred heterologous genes and their environmental biosafety.

P-1247

Gamma Radiation-Induced Banana Bud Mutants *In Vitro* Salt Tolerant. LUIZ G. B. FERRAZ, Hélio A. Burity, Waldeciro Colaço, Luiza S. S. Martins, Terezinha R. Câmara, Nara S. A. Freitas, Maria A. J. F. Ferreira, L. G. Willadino, and Maria A. G. Barbosa. Pernambuco State Agricultural Research Company. Recife—PE, Brazil. P.O.B. 1022. E-mail: bione@ipa.br

The soil salinity is one of the most serious problems for the world agriculture, mainly in semiarid region as in the Brazilian Northeastern where the total area affected by salts is over 3 millions hectares. In Brazil 624 thousand hectares are cultivated with bananas. The cv. Pacovan is largely cultivated and it is known as highly sensitive to salt stress. The development of banana genotypes tolerant to salinity through conventional breeding is problematic due to the low seed production and to their partial or total sterility. In preliminary essays it was possible to evaluate the sensibility of the 'Pacovan' banana buds to the NaCl and to the gamma irradiation. At the concentration of 100 mM of NaCl, there was a decrease of 40 % in the bud surviving and the surviving individuals did not regenerate or produce any lateral buds, and all explants died at 200 and 250 mM of NaCl. The gamma radiation did not reduce significantly the bud surviving but decreased the multiplication and the dry matter production. The selection of mutants included a pre-selection phase (multiplication) and an effective selection stadium (rooting) both under 100 mM salt. At pre-selection the surviving of the 480 irradiated buds was drastically reduced, with 20 lines have been pre-selected. At the final, 17 lines were selected. Among the isoenzyme systems used to characterize the genotypes (control and mutants), the Peroxidase, Esterase and Glutamate Oxalacetate Trasaminase presented different enzimatic activity among individuals, while Acid Phosphatase presented no difference. Alcohol Desidrogenase and Shikimate Desidrogenase presented no enzimatic activity. No polymorphism could be detected after the RAPD analysis. Some phenotypic and morphogenetic alterations and aberrations could be observed in the mutants.

P-1248

Salinity Tolerance/Toxicity of Different Citrus Cell Cultures. ANA LUÍSA FERREIRA and M. Emilia Lima-Costa. University of Algarve, Faculty of Engineering of Natural Resources. Campus of Gambelas, 8000–117 Faro, Portugal. E-mail: alferrei@ualg.pt

High salt concentration in the soil solution is one of the most important constrains to crop growth around the world. Plant growth response to salt *stress* follows a complex pattern as the result of both, the low water potential in the soil and the build up of toxic salts in the plant tissues. The behaviour to osmotic stock between two cultivars of *Citrus* in order to approach the cell and molecular mechanism of salt tolerance in these species was investigated. *Citrus* cell lines of both cultivars were grown in Murashige and Tucker [1] medium supplemented with NaCl at concentration between 0 to 400mM. *Citrus* cv. Carvalhal cell line exhibited a cell resistance feature, evidenced by a constant intracellular proline content and a slight impact on the biomass parameters along the NaCl gradient was showed. Furthermore, the fresh weight increase, at the 400mM extreme NaCl content, was only reduced to half of its initial value. On the other hand, *Citrus* cv. Valencia showed, apparently, no tolerance to saline stress and cell toxicity was evidenced at all salt conditions tested, throughout a strong reduction on the fresh weight increase and the increase (2-fold) in the proline intracellular content. Cell death was observed for this cell line at NaCl content, higher than 150mM. Selection of cell lines for high salt tolerance, *in vitro*, can be relevant, in order to develop more salt tolerant citrus types. [1] Murashige T. and Tuker D.H.P. (1969)—Growth factor requirements of *Citrus* tissue culture. In: H Chapman, ed, Proceedings of the First International *Citrus* Symposium, Vol 3, University of California, Riverside, pp 1151–1161.

P-1249

Banana Genetic Transformation by Biolistic and Plant Regeneration from Embryogenic Callus. L. H. Kido, M. C. Falco, E. A. Kido, M. C. Silva-Filho, A. Tulmann-Neto, A. FIGUEIRA. Centro de Energia Nuclear na Agricultura and Escola Superior de Agricultura Luis de Queiroz, Universidade de São Paulo, CP 96, Piracicaba, São Paulo, 13400–970, Brazil. e-mail: figueira@cena.usp.br

Banana is an important staple food in the tropics, and a highly appreciated fruit in temperate countries. Brazil is a major producer, and banana cultivars from subgroups "Pomme" and "Silk" (genome AAB) are widely cultivated, mostly by small growers, and locally consumed. Breeding is based on the development of tetraploid hybrids derived from crosses between a few triploid cultivars and wild diploids. However, most of the triploid bananas are sterile, limiting the possibility of hybridization. Genetic transformation, based on biolistic or *Agrobacterium* vector, is an essential tool for genetic improvement of commercial triploid cultivars, but published protocols have mainly focused on export-type "Cavendish" bananas (genome AAA). Further, there is no efficient callus regeneration method for the cultivars important in Brazil. This work aimed to optimize a method of plant regeneration from callus derived from cell suspension cultures from male inflorescences of cultivar "Maçã" ("Silk" type), and a transformation protocol using microparticle bombardment of this embryogenic callus. Calli were bombarded with various plasmid constructions, differing for *uidA* (GUS) promoter, and kept in the dark, on embryo induction media for 6 months, subculturing onto fresh media every 30 days. Transient expression was confirmed by GUS histochemical assay one week after bombardment. All constructs gave GUS-positive, with similar levels of expression from maize ubiquitin or the duplicated 35S CaMV promoter. After initial embryo development, calli were transferred to regeneration media under light for plantlets growth. Plantlets were regenerated from all materials. Calli transformed with pBI426 (*uidA* and *neo*) gave the largest number of regenerated plants with superior leaf and root growth. Based on the optimized protocol, experiments are underway to introduce gene constructs to reduce plant height, a major problem with traditional cultivars and new tetraploid hybrids. Financial support: FAPESP 97/10969–4; 00/12937–7; CNPq.

P-1250

Comparison of GFP and GUS Selectable Marker Gene Expression in Explants of Peach and Plum. AGNIESZKA GOLIS[1], Isabel M. G. Padilla[2], Adele Gentile[3], Carmine Damiano[3], and Ralph Scorza[2]. [1]Research Institute of Pomology and Floriculture, Skierniewice, Poland; [2]USDA-ARS Appalachian Fruit Research Station, Kearneysville, WV 25443; and [3]ISF, Dept of Propagation, Rome. E-mail: agolis@afrs.ars.usda.gov

The development of transgenic plants is aided by the ability to visually select transgenic cells and regenerated shoots. GUS has been commonly used for the *in vitro* evaluation of transformation in herbaceous and woody species. The disadvantage of the GUS system is the destructive nature of the test. In order to facilitate the transformation of peach and plum, the GFP selectable marker gene was tested. We compared the expression of GFP and GUS in plum hypocotyl slices and in different explants of peach used for regeneration and transformation studies. GUS expression was 45% in 'Bluebyrd' plum hypocotyl sections with 25% of explants showing very high expression and 20% with an average of 4.6 spots per explant. GFP was expressed in 82% of 'Bluebyrd' hypocotyl sections with 87% of GFP positive sections showing high expression (>15 spots/explant) and 13% with low expression (<5 spots/explant). 'Bailey' peach hypocotyl sections expressed GUS in only 7% of explants (2 spots/explant) and no explants expressed GFP. In this peach cultivar 3% of internodes expressed GUS (1.2 spots/explant) and 10% expressed GFP (2.3 spots/explant), in leaves 2% expressed GUS (2 spots/explants) and 4% expressed GFP (3.5 spots/explant). Studying GFP expression in 9 peach cultivars and three explant sources we found no expression in hypocotyl sections for any cultivar, 0–35% in cotyledons (0–26 spots/explant) and 0–28% in internodes (0–5 spots/explants), depending on the cultivar. Our results indicate that both GUS and GFP are highly expressed in plum hypocotyl slices. Low expression of both markers was seen in all peach explants. The low expression in peach was likely due to low rates of transformation. In this case, GFP may be more useful than GUS as a marker system for peach because it would allow for the more rapid and non-destructive evaluation of transformation and the early selection of transgenic cells that could then be proliferated and exposed to regeneration treatments.

P-1251

Induction of Systemic, Broad Spectrum Disease Resistance in Grapevine by In Vitro Selection. S. Jayasankar, Zhijian Li, D. L. Hopkins, and D. J. GRAY. Mid-Florida Research and Education Center, Institute of Food and Agricultural Sciences, University of Florida, 2725, Binion Road, Apopka FL 32703. Email: djg@mail.ifas.ufl.edu

Embryogenic suspension cultures of grapevine (*Vitis vinifera* L.) 'Chardonnay' were selected with phytotoxic culture filtrate produced by *Elsinoe ampelina*, the causal agent of anthracnose disease. Regenerated plants that exhibited resistance to anthracnose (Jayasankar et al., 2000, Planta, 211:200–208) were planted in the vineyard and grown for three years. The seven most vigorous vines from a total population of 96 were cloned and tested for resistance to *Xylella fastidiosa*, a xylem-limited bacterium that causes Pierce's disease (PD). One of the seven vines exhibited only mild PD symptoms after repeated artificial inoculations, during which time all other selected and control vines were killed. Protein profiles from xylem sap also revealed differences between this vine and the others. These results suggest the induction of systemic, broad spectrum resistance in grapevine after *in vitro* selection with a fungal culture filtrate.

P-1252

Sucrose Level in the Shoot Multiplication Medium Influences Shoot Proliferation and GUS Expression in Leaves from In Vitro Propagated Blueberry Shoots. F. A. HAMMERSCHLAG, X. Cao, and I. Fordham. USDA/ARS, Fruit Laboratory, Building 010A, BARC-W, Beltsville, MD 20705. E-mail: HAMMERSF@BA.ARS.USDA.GOV

As part of a program to develop protocols aimed at improving highbush blueberry (*Vaccinium corymbosum* L.) via either genetic engineering or tissue culture techniques, studies were conducted to examine the effects of sucrose concentration in the shoot multiplication medium on in vitro shoot proliferation and on the transfer of an intron-containing Beta-glucuronidase (GUS) gene into leaf explants from the in vitro propagated shoots. Shoot (> 0.5 cm) production from cultivars Bluecrop, Duke and Georgiagem increased 1.3 to 1.8-fold, 1.4 to 1.9-fold and 1.3 to 2.3-fold when the sucrose concentration was increased from 15 mM to either 29, 44 or 58 mM, respectively. Four days of cocultivation with *Agrobacterium tumefaciens* strain EHA105 yielded maximum GUS-expressing leaf zones on leaf explants from shoots cultured on either 15 or 29 mM sucrose. The number of GUS-expressing leaf zones was significantly less on leaf explants from shoots on 58 mM sucrose than from those on 15 mM sucrose for all three cultivars. These studies suggest that shoot pretreatment conditions need to be considered for optimizing subsequent blueberry genetic engineering experiments. Thus, a multiplication medium containing 29 mM sucrose is recommended to obtain both high levels of shoot production and GUS expression with cultivars Bluecrop and Georgiagem, but 15 mM sucrose is recommended for cultivar Duke.

P-1253

The Use of Microsatellites to Investigate the Zygosity State of Apple Plants Obtained by Methods of Haploid Induction. M. HÖFER[1], A. Gomez[2], M. A. Bueno[2], E. Aguiriano[2], and J. A. Manzanera[3]. [1]Federal Centre of Breeding Research on Cultivated Plants, Institute of Fruit Breeding, Pillnitzer Platz 3a, D-01326 Dresden, Germany; [2]INIA-CIFOR Madrid, Spain; and [3]IMIA, Apdo., Spain. E-mail: M.HOEFER@BAFZ.DE

Haploids in apple were induced using anther and microspore culture and *in situ* parthenogenesis followed by *in vitro* culture of immature embryos or cotyledons. Flow cytometrical analysis has shown a distribution of the ploidy level between the embryos and shoots of one donor genotype from the haploid to the tetraploid state and so the investigation of the homozygosity was of top priority. In addition to an earlier characterization of the zygosity state using isozymes, the purpose of these experiments was an analysis of SSR (Simple Sequence Repeats) to describe the homozygosity more comprehensively. Five SSR primer combinations previously described by Guilford et al. (1997) were applied. Initially, PCR amplification with the primer pairs was performed on the donor genotypes of haploid induction and on three further apple cultivars. Only two primer pairs which amplified two different contrasting alleles could be used for investigation of the zygosity state. The analysis of anther-derived lines by SSR markers corroborates the previous results obtained by isozymes, all samples showing only one allele from the donor genotype. This indicates that all have a haploid origin although they are polyploids now. Concerning *in situ* parthenogenesis, no heterozygous diploid samples have been detected previously by any of the tested markers, which implies that there was no pollination, and the nonexistence of non–specific alleles is proof of the haploid origin of the *in situ* parthenogenic lines analyzed.

P-1254

A Transgenic Approach to Improve the Digestibility of Sorghum Grain. LIZ IZQUIERDO, S. J. Gray, and I. D. Godwin. School of Land and Food Sciences, University of Queensland, Brisbane, QLD 4072. Australia. E-mail: liz.izquierdo@mailbox.uq.edu.au

Sorghum (Sorghum bicolor) grain is an important staple food in some semiarid tropical countries. However, it has lower protein and starch digestibility than maize, wheat, rice, and barley, which makes it less competitive on the world market. The nature and the way storage proteins are distributed within the endosperm of the sorghum seed are important in determining the low digestibility of the grain. One of these proteins, gamma kafirin, is highly disulphide bound and protease resistant. This protein is located on the periphery of the protein bodies (starch:protein matrix) impeding the access to the other more digestible kafirins (alpha and beta) and starch. We aim to downregulate the levels of expression of this protein through genetic engineering. A cDNA clone of the gene encoding gamma kafirin was obtained from RNA of immature seeds of sorghum and inserted into an expression vector in sorghum using microprojectile bombardment approach. We aim to produce transgenic plants carrying an inverted repeat of the coding sequence of this gene under a cereal endosperm specific promoter driving its expression.

P-1255

Transgenic Bread and Durum Wheat Lines that Do Not Express the High Molecular Weight Glutenin Gene Family are Useful for Studies in Cereal Chemistry. MARK C. JORDAN[1], S. Cloutier[1], C. Rampitsch[1], O. Lukow[1], A. Benabdelmouna[2], K. Armstrong[2]. [1]Cereal Research Centre, Agriculture and Agri-Food Canada, 195 Dafoe Rd., Winnipeg, MB, R3T 2M9, Canada and [2]Eastern Cereal and Oilseed Research Centre, Agriculture and Agri-Food Canada, Ottawa, ON, K1A 0C6, CANADA. E-mail: mcjordan@em.agr.ca

End use functionality of wheat depends on the protein content and composition of the grain. Contributing factors include the presence of particular subunits of high and low molecular weight glutenin, the ratio of high molecular weight glutenin to low molecular weight glutenin and the ratio of glutenin to gliadin. Transformation allows these factors to be studied within a particular background thus contributing to our understanding of end use functionality. The genes encoding the high molecular weight glutenin subunits Dx5 and Dy10 were used to transform bread and durum wheat. After transformation bread wheat and durum wheat lines exhibiting silencing of all high molecular weight glutenins were produced. Molecular cytogenetic analysis of these lines indicated the presence of one large multi-copy locus with transgene DNA interspersed with plant DNA. As the locus was simply inherited homozygous progeny could be isolated and increased for rheological studies. These lines provide a "blank slate" in which to study the impact of high molecular weight glutenins on wheat end use functionality. The null lines do not differ in total protein content from control lines and preliminary evidence suggests they have similar amounts of insoluble glutenin (which is thought to be primarily determined by HMW glutenin subunits). Properties of wheat flour that are mainly tests of gluten strength were significantly different from control lines thus confirming the role of HMW glutenins in conferring gluten strength.

P-1256

Introduction of A Citrus Blight Associated Gene into Carrizo citrange [*Citrus sinensis* (L.) Osbc. x *Poncirus trifoliata* (L.) Raf.] by *Agrobacterium tumefaciens*. M. KAYIM (1), T. C. Ceccardi (2), M. J. G. Berretta (1), G. A. Barthe (1), and K. S. Derrick (1). (1) University of Florida, Dept. of Plant Pathology, CREC, 700 Experiment Station Road, Lake Alfred FL. 33850 and (2) Celera Functional Genomics, South San Francisco, CA. Email: KAY@LAL.UFL.EDU

To determine the effect over or blocked production of citrus blight associated protein p12 might have on disease symptoms, sense and antisense constructs of the p12 gene were transferred to Carrizo citrange using an *Agrobacterium*-mediated transformation system. Longitudinally cut epicotyl segments,1–1.5 cm lenght from *in vitro* grown seedlings were co-cultivated with either *A. tumefaciens* strain, EHA-101 carrying a binary vector pGA482GG/WP12 and strain Agl-1 carrying a binary vector pCAMBIA2301/WaP12 separately. Approximately 500 putative transgenic shoots regenerated from 200 explants using kanamycin at 50 mg/l as a selective agent. Two hundred putative transgenic shoots, 45 sense 155 antisense were assayed histochemically for ß-glucuronidase (GUS) activity. Thirty sense and sixty two antisense transgenic shoots were positive. GUS positive shoots were *in vivo* grafted onto Carrizo citrange rootstocks for further analyses. Two months after grafting, plants were analysed by PCR for GUS, NPT II, p12 sense and antisense genes. All GUS positive plants showed p12 sense (without the intron associated with the gene in untransformed citrus) and antisense genes. Six months after grafting plants were analysed by Southern hybridizations and western blots.

P-1257

High Transformation Efficiency and Increased Transgenic Plant Regeneration in Citrus sp. by the Combination of *Agrobacterium tumefaciens* and Plasmolysis Treatment. M. KAYIM, G. A. Barthe, M. J. G. Beretta, and K. S. Derrick. University of Florida, Department of Plant Pathology, CREC, 700 Experiment Station Road, Lake Alfred, FL 33850. E-mail: KAY@LAL.UFL.EDU

We have developed procedures for high efficiency production of transgenic citrus plants using an *Agrobacterium tumefaciens* system with plasmolysis treatment. Longitudinally cut epicotyl segments of eight different citrus species [Milam rough lemon (*Citrus jambhiri* Lush.), Volkamer lemon (*Citrus volkameriana* L.), rangpur lime (*Citrus limonia* L.), 'Hamlin' sweet orange (*Citrus sinensis* L. Osbeck), 'Duncan' grapefruit (*Citrus paradisi* Macf.), sour orange (*Citrus aurantium* L.), Cleopatra mandarin (*Citrus reticulata* Blanco) and Carrizo citrange (*Citrus sinensis* L. Osbeck x *Poncirus trifoliate* L. Raf.)] were plasmolyzed in different concentrations of sucrose and maltose [0, 3, 6, 8, 9, 10, 12% (w/v)] prior to *Agrobacterium* inoculation. Plasmolyzed epicotyl explants were cocultivated with either the hypervirulent *Agrobacterium tumefaciens* strain, the EHA-101 (harboring a binary vector pGA482GG) or Agl-1 (carrying pCAMBIA1303 vector). Both binary vectors contained neomycin phosphotransferase II (NPT II) and β–glucuronidase (GUS) genes. The binary vector pCAMBIA1303 also contained a fused mGFP5 gene at the 3′ end of GUS gene as a reporter. Epicotyl explants of rangpur lime, rough and Volkamer lemons plasmolyzed in 6–10% maltose showed transient GUS gene expression comprising up to 95% of the cut surface of explants, while Carrizo citrange showed 80% expression when they were plasmolyzed in 6–10% sucrose. On the other hand, epicotyl explants of sweet orange, grapefruit, sour orange and Cleopatra mandarin showed transient GUS expession in 80–90% of explants. Stable transformation frequencies were 120–143% for Carrizo citrange, 90–110% for grapefruit, 80–85% for sweet orange, 30–70% for sour orange, rangpur lime, Milam rough and Volkamer lemons. Regenerated putative transgenic shoots were harvested from the cut surface of epicotyl explants within 2–3 months. Shoots were divided into basal and apical portions. Basal portions were assayed for GUS and apical portions were shoot tip grafted *in vivo* for the production of whole plants. The presence and expression of transgenes in the whole plants were verified by GUS assay, PCR and Southern analyses. The transformation efficiencies in different species obtained are the highest so far repoted for citrus.

P-1258

Manipulations of the Flavonoid Pathway in *Citrus* to Have Better Acceptability of Fruits. UFUK KOCA 1, Mark Berhow 2, Yoram Eyal 3 and Gloria A. Moore 1. 1-Horticultural Sciences, Plant Molecular and Cellular Biology Program, University of Florida, Gainesville FL 32611; 2-USDA-REE-ARS-MWA-NCAUR-BAR, Peoria, IL 61604; and 3-Volcani Institute Bet Dagan, Israel. E-mail: UKOCA@MAIL.IFAS.UFL.EDU

Flavonoids are a widely distributed, diverse group of phytochemicals. In citrus, flavanone disaccharides are the main group of flavonoids that accumulate, and may affect the taste of fruits. Specifically, flavanone neohesperidosides, for instance naringin, cause bitterness in fruit and fruit juice products. This bitterness of the fruit reduces the acceptability of the fresh fruit and juice products in commercial markets. Our main objective is to manipulate the production of flavanone neohesperidosides in citrus using molecular genetics and transformation techniques. As an initial step, we have isolated the cDNAs of chalcone synthase (CHS) and chalcone isomerase (CHI) genes. Sense and antisense constructs of these cDNAs were used to transform grapefruit seedlings to suppress the expression of the target gene or increase the nonbitter flavonoids. To accomplish our goal we also transformed grapefruit with cDNA of a hairpin-forming construct of the 1–2 rhamnosyl transferase gene, which catalyzes the last biochemical step in the formation of naringin. The construct was designed to express dsRNA, which interferes with the target gene activity. Transgenic plants are being analyzed by HPLC for their flavonoid composition and, are being characterized for their target gene copy numbers.

P-1259

The Influence of Two Strains of *Agrobacterium* on the Efficiency of Apple Transformation. SERGEI F. KRASNYANSKI and Schuyler S. Korban. Department of Natural Resources & Environmental Sciences, University of Illinois, Urbana, IL 61801. E-mail:ksergei@staff.uiuc.edu

In order to optimize the efficiency of *Agrobacterium*-mediated transformation in apple (*Malus x domestica* Borkh.) cv. Gala, the effect of two strains of *Agrobacterium*, KYRT-1 and GV3101, was evaluated. Both strains carried the same binary vector encoding *npt* II and *uid*A genes. Young leaves from in vitro-grown shoot cultures were used as explants. Following inoculation and cocultivation with *Agrobacterium*, explants were cultured on a regeneration medium containing 100 mg/L kanamycin and 500 mg/L cefotaxime. After four weeks of culture, up to 45% of explants inoculated with KYRT-1 formed callus, 5 to 30 calli per explant, stained positive for GUS. Whereas, only 10% of leaf explants inoculated with GV3101 formed GUS positive callus. Regeneration of putative transformed shoots from explants inoculated with *Agrobacterium* strain GV3101 was observed via direct organogenesis without any callus phase. However, no shoot primordia were observed on leaves transformed with KYRT-1 *Agrobacterium* strain. The implications of these finding will be discussed further.

P-1260

A Novel 11 kD Methionine-rich Protein from Maize Endosperm for Use in Transgenic Soybeans. WONSEOK KIM and Hari B. Krishnan. Plant Genetics Research Unit, United States Department of Agriculture-ARS, University of Missouri, Columbia, MO 65211. E-mail: KrishnanH@missouri.edu

Soybean *(Glycine max [L.] Merr.)* is a rich source of protein for livestock and humans. However, the nutritional quality of soybean proteins is not optimal due to a deficiency in sulfur-containing amino acids. One-way to overcome this problem is by expressing heterologous proteins in soybean that are extremely rich in sulfur-containing amino acids. Brazil nut 2S albumin, maize delta-zeins, and synthetic proteins rich in methionine and lysine have been expressed in transgenic crops with varying amount of success. We are interested in expressing high-methionine delta-zeins in soybeans to elevate the methionine content of soybean seeds. As a first step in this direction, we have isolated genes encoding the delta-zeins. In addition to the previously identified 10 and 18 kD delta-zeins, we identified a novel 11 kD methionine rich delta-zein from developing endosperm of the W23a1 maize inbred line. The nucleotide sequence of this new delta-zein is identical to the published 10 kD delta-zein except for an insertion of 18 nucleotides between +316 and +333 bp from the translation start site. The 11 kD zein contains 21% methionine. Northern blot analysis revealed temporal differences in the RNA transcript levels of the 11 kD and 18 kD delta-zeins between B73 and W23a1 maize inbred lines. Western blot analysis using antibodies raised against the purified 11 kD zein showed gradual accumulation of this protein throughout the seed development. Electron microscopic immunocytochemistry indicated that the 11 kD delta-zein was localized in the protein bodies. Experiments are in progress to express this novel methionine-rich protein in transgenic crops.

P-1261

Developmental Regulation of Peach ACC Oxidase-GUS Fusions in Transgenic Tomato Fruits. HANGSIK MOON and Ann M. Callahan. U.S. Department of Agriculture, Agricultural Research Services, Appalachian Fruit Research Station, 45 Wiltshire Road, Kearneysville, WV 25430. E-mail: HMOON@AFRS.ARS.USDA.GOV

Fruit ripening involves changes in the expression of a large number of genes including the well-characterized 1-aminocyclopropane-1-caboxylic acid (ACC) oxidase which catalyzes the conversion of 1-aminocyclopropane-1-caboxylate to ethylene. We isolated a genomic DNA sequence (*PpACOI*) encoding ACC oxidase from peach (*Prunus persica* L. Batsch) that has three introns and 2.9 kb of 5' flanking region to the start codon. Previous work with the related cDNA had shown that the accumulation of the mRNA occurred during the softening stage of fruit ripening and that this phenomenon was associated with increases in the amount of ethylene synthesized by fruit (Callahan et al., Plant Physiol., 100: 482–488). To investigate the regulation and tissue specificity of the peach ACC oxidase gene expression, chimeric fusions between 403, 610, 901, 1319, 2141 and 2913 bp, respectively, of the 5' flanking region of the *PpACOI* sequence and the β-glucuronidase (GUS) coding sequence were constructed and used to transform *Lycopersicon esculentum* (Mill cv. Pixie). Tomato plants transformed with pBI121 (CaMV 35S-GUS) and pBI101 (GUS with no promoter) were positive and negative controls, respectively. Histochemical GUS analysis of the transgenic tomato fruits indicated that the 403 bp of the 5' UTR is sufficient to confer significant GUS activity. The 2913 bp of *PpACOI* upstream sequence directed GUS expression in the early green stage of fruit development, and increased GUS activity as fruit matured, reaching maximum at 'pink' or 'light red' stage. There was little or no GUS staining in leaves or stems. The 2141 bp promoter drove GUS expression from the early green stage as did the 2913 bp promoter, but the GUS activity decreased at late stages of fruit development indicating a regulatory region between −2913 and −2141. There appear to be at least one positive and one negative regulatory region between −2913 bp and −403 bp of *PpACOI* that influence GUS expression at different fruit developmental stages.

P-1262

Characterization of the Maize Glutamine Synthetase$_{1-2}$ Promoter. M. J. MUHITCH, H. Liang[1], R. Rastogi, and K. G. Sollenberger. Mycotoxin Research Unit, National Center for Agricultural Utilization Research, ARS/USDA, 1815 N. University St., Peoria, IL 61604 and [1]Present address: Dept. Molecular Genetics & Cell Biology, University of Chicago, 1103 East 57th Street, Chicago, IL 61637. E-mail: muhitchm@ncaur.usda.gov

Glutamine synthetase (GS), plays several central roles in plant nitrogen metabolism including initial incorporation of inorganic nitrogen, nitrogen recapture in photo-respiration and nitrogen mobilization in senescence and seed fill. In maize, GS is encoded as a single plastid gene and five cytoplasmic genes. The protein (GSp1) derived from the cytoplasmic gene GS1–2 is found in abundance within the pedicel and surrounding pericarp of developing seeds and is believed to be an important regulatory enzyme in nitrogen assimilation into developing maize kernels. This gene has now been isolated, its 5' upstream regulatory region has been sequenced and a 664 bp upstream region used to express b-glucuronidase (GUS) in stably transformed maize plants. GUS expression was very strong in the basal maternal seed tissues, including the surrounding pericarp and was also evident in the bases of developing anthers, but did not accumulate in leaves, roots, endosperm or embryo tissues. GUS expression was also noted in pollen in some lines, despite GSp1 not being found in pollen. Deletion series analysis of the promoter indicates that a sequence between −664 and −400 confers maternal tissue specificity. The second intron also appears essential as its removal abolishes tissue specificity as well. The GS1–2 promoter's strong expression in maternal seed tissues is consistent with the GS isozyme's role in nitrogen metabolism during grain fill. The promoter could be used to modify carbon and nitrogen assimilation into developing kernels or to add disease resistance factors into critical pericarp and pedicel tissues.

P-1263

Detection of Genetic Variations in Tissue Culture-derived Lemon Plants Using RAPD and Flow Cytometry. Z. VILORIA, M. Calovic, B. Nielsen, V. Orbovic, F. G. Gmitter, Jr., W. Castle, and J. Grosser. Citrus Research and Education Center—University of Florida, 700 Experiment Station Road, Lake Alfred, FL 33850. E-mail: VILORIA@LAL.UFL.EDU

Five populations of lemon plants obtained from immature ovules through different tissue culture procedures were examined for the presence of somaclonal and irradiation-induced genetic variation. The first group of plants represented in-vitro grown nucellar seedlings that served as a source from which the second group of plants were derived via organogenesis from internodal stem sections. The third group of plants was obtained via somatic embryogenesis from nucellus-derived embryogenic callus. The fourth group of plants was obtained the same way as the third except that the embryogenic callus was irradiated with gamma rays prior to induction of embryogenesis. The last group of plants was regenerated from protoplasts that were isolated from nucellus-derived embryogenic suspension cultures. DNA samples from plants of all 5 groups were screened for polymorphism among RAPD fingerprints amplified by ten 10-mer primers. Flow cytometry was performed using macerated leaf tissue stained with DAPI. Among all tested plants, genetic variation was detected only within the group of plants recovered from irradiated embryogenic calli. Out of 72 plants from that group, three had RAPD fingerprints different from the rest of the population. One plant was found to be cytochimeric, consisting of diploid and tetraploid cells.

P-1264

Effect of Explant Source on Regeneration and Transformation Efficiency in Galia Melon (*Cucumis melo* L.). HECTOR G. NUNEZ-PALENIUS, Daniel J. Cantliffe, and Harry Klee. Horticultutal Sciences Department, Univ. of Florida, IFAS, Gainesville, FL 32611-0690. Email: hgnunez@ufl.edu, djc@ufl.edu, hjklee@gnv.ifas.ufl.edu

Different Plant organs such as; shoot apex (Adelberg *et al.*, 1999), cotyledon (Guis *et al.*, 1997), root (Kathal *et al.*, 1994), hypocotyl (Moreno *et al.*, 1985), petiole (Punja *et al.*, 1990) and leaf (Yadav *et al.*, 1996) have been used as explants for melon *in vitro* culture. All the systems had diverse regeneration rates depending on culture conditions, cultivar and explant source. The goal of this work was to measure the source of the explant on regeneration and transformation efficiency in Galia melon. Gaba *et al.* (1999) reported Galia melon to be recalcitrant to transformation by *Agrobacterium tumefaciens*. Cotyledon, hypocotyl and true-leaves of both female and male Galia parental line were used for transformation. The ABI strain of *Agrobacterium tumefaciens* carrying a plasmid vector containing a construct harboring the GUS gene under the constitutive promoter 35S and a glyphosate tolerance gene as selectable marker was used. Hypocotyls were transformed according the protocol described in Ramirez-Malagon & Ochoa-Alejo (1996). We applied a protocol developed in our lab to transform cotyledons (Nunez-Palenius *et al.*, 2001). Using the methodology by Guis *et al* (2000) true-leaf explants were transformed. The greatest numbers of shoots were regenerated from cotyledons compared with hypocotyl and true-leaf explants. Plants regenerated from cotyledons also developed the highest number of GUS positive shoots and roots. We were not able to obtain any transgenic shoots using true-leaves as explants. According with our results, Galia melon is readily transformable with *Agrobacterium tumefaciens* using the cotyledon-protocol.

P-1265

Molecular Indexing for the Huanglongbing Disease of Citrus. S. D. OBUKOSIA, E. Mutitu, Kimani Waithaka, F. Olubayo, and T. Magomere. Departments of Crop Science and Crop Protection, University of Nairobi, PO Box 29053, Nairobi, Kenya. Email: citrustc@wananchi.com

Prior to procurement of citrus germplasm by importation or sale of citrus planting materials to farmers from commercial nurseries, there is need to index for the huanglongbing disease of citrus. The huanglongbing disease is caused by the phloem-limited bacterium belonging to the species *Candidatus* Liberobacter of the alpha subdivision of the class *Proteobacteria*. This disease is currently a threat to the survival of the citrus industry worldwide, sometimes causing yield losses of up to 100%. In this study, citrus seedlings/plants from tissue culture, nurseries, and fields were indexed using the polymerase chain reaction with specific primers for the 16S rDNA of the *Candidatus* Liberobacter using the forward primer (5'GCGCGTATGCAATACGAGCGGCA3') specific for the *Candidatus* Liberobacter africanum and reverse primer (5'GCCTCGCGACTTCGCAACCCAT3'). Plants from tissue culture supported nurseries were HLB-disease free. Plants from high altitude nurseries/fields (1000 m above sea level) and high altitude (2000 m) not supported by tissue culture were infected with the *Candidatus* Liberobacter africanum giving a PCR fragment of about 1100 basepairs. The indexing technique is currently used to support a commercial nursery for the production of HLB-disease free citrus seedlings for farmers through tissue culture.

P-1266

Genetic Transformation of *Citrus paradisi* cv. Flame with the Coat Protein of Three Isolates of *Citrus* Tristeza Closterovirus. V. ORBOVIC[1], N. Noveski[1], G. A. Moore[2], and J. W. Grosser[1]. University of Florida-IFAS, [1]-Citrus Research and Education Center, Lake Alfred, FL 33850; [2]-Department of Horticultural Sciences, Gainesville, FL 32611. E-mail: orbovic@lal.ufl.edu

Genetically transformed plants of grapefruit cv. Flame have been obtained in the procedure employing *Agrobacterium tumefaciens* strain EHA101. All Agrobacteria used in these experiments, harbored binary vector pGA482GG with β-glucuronidase as a reporter gene, but differed according to presence of genes for coat proteins of three different isolates of Citrus Tristeza Closterovirus (CTV): B249, T30, and T36. Explants of epicotyl segments were incubated in bacterial suspension and left on selective regeneration medium (RM) for 4–5 weeks until shoots appeared and were 1–5 mm in size. These shoots were removed from explants and put onto growth medium (GM) for additional 4 weeks. After this period, small tissue sample was taken from each shoot and used for GUS assay. Only those shoots that exhibited blue staining in GUS assay were placed onto root induction medium (MSR). Roots have developed within 4 weeks and were allowed to grow for additional 4 weeks before the whole plants were transferred to soil. In the other experiment, shoots were micro-grafted in vitro after 4 weeks growth period on GM. Experiments are under way to confirm stable insertion of T-DNA into the genome of plants that exhibited blue staining in GUS assay. Resistance of these plants to CTV will also be evaluated.

P-1267

An Improved Method for Routine Transformation of Plum (*Prunus domestica* L.). ISABEL M. G. PADILLA, Kevin Webb, and Ralph Scorza. USDA-ARS Appalachian Fruit Research Station, Kearneysville, WV 25443. E-mail: Ipadilla@afrs.ars.usda.gov

We describe a system for routinely transferring genes into plum (*Prunus domestica* L.) through the use of *Agrobacterium tumefaciens*. This method is based on regeneration of shoots from plum hypocotyl sections from ungerminated mature seeds that may be used fresh or cold-stored for up to 3 years. Shoot regeneration medium was based on Murashige and Skoog salts at 3/4 strength with 7.5 μM thidiazuron and 0.25 μM indolebutyric acid. Delayed kanamycin (kan) selection or immediate selection was compared in these tests. After 2 days of co-cultivation explants were transferred to the regeneration medium with 80 mg/l of kan and 300 mg/l timentin or to regeneration medium with 300 mg/l timentin for 2 weeks before exposure to kan. Immediate transfer to kan-containing medium reduced escapes by 80 percent without reducing the total number of transformed shoots recovered. Transformation rates using either delayed or immediate selection systems were 1–9% of hypocotyl slices producing transgenic plants although the delayed selection systems required more time and effort to eliminate non-transformed escapes. The transgenic shoots rooted at a 90% rate on half-strength Murashige and Skoog salts with 5.0 μM naphthalene-acetic acid and 0.01 μM kinetin. Plantlets were transferred to the greenhouse directly from culture tubes with a 90% average survival. Gene insertion was confirmed by DNA blotting. With this system we have produced transgenic plants containing the prune dwarf, Prunus necrotic ringspot and tomato ringspot virus coat-protein genes.

P-1268

Towards Genetic Improvement of *Citrus* Through Molecular Breeding. L. PEÑA, M. Cervera, R. Ghorbel, A. Domínguez, C. Fagoaga, J. Juárez, J. A. Pina, and L. Navarro. Department Plant Protection and Biotechnology, Instituto Valenciano de Investigaciones Agrarias, Apartado Oficial, 46113-Moncada, Valencia, Spain. E-mail: lpenya@ivia.es

Citrus is the most economically important fruit tree crop in the world. Genetic improvement of citrus through conventional breeding is limited by their genetic and reproductive characteristics, with many cases of cross- and self-incompatibility, high heterozigosity, apomixis, very long juvenile periods, and lack of knowlege of the mode of inheritance of the most important horticultural traits. Genetic transformation can be used as a powerful tool to improve citrus rootstocks and varieties. We have established genetic transformation systems for citrus species and hybrids, which are allowing us now to incorporate transgenes of potential agricultural interest into them. Some of our research lines are the following: 1, we are introducing meristem-identity transgenes into citrus with the aim to shorten the juvenile period and consequently to accelerate genetic improvement programs; 2, we are introducing transgenes into citrus to get higher tolerance or resistance against diseases caused by oomycetes (as *Phytophthora* sp.) and viruses (as citrus tristeza virus); and 3, we are introducing transgenes involved in hormonal biosynthetic pathways into citrus to try to modulate tree size. Furthermore, we are conducting a field trial with transgenic citrus plants to investigate phenology of trees, transgene stability, frequency of transgene dispersion through the pollen, and transgene inheritance to the progeny. Recent developments from all these projects will be presented.

P-1269

Effects of Pectate Lyase and Cellulase Genes, in Antisense Expression, on Modification of Strawberry Fruit Softening. F. PLIEGO-ALFARO[1], S. Jimenez-Bermudez[2], J. Muñoz-Blanco[3], J. L. Caballero-Repullo[3], L. Trainotti[4], G. Casadoro[4], V. Valpuesta[1], M. Barceló-Muñoz[2], M. Cordero de Mesa[1], M. A. Quesada-Felice[1], and J. A. Mercado-Carmona[1]. (1) Departamento de Biología Vegetal, Campus de Teatinos, s/n, 29071 Málaga, Spain; (2) CIFA, 29140 Churriana, Málaga, Spain; (3) Dpto. de Bioquímica y Biología Molecular, Universidad de Córdoba, 14071 Córdoba, Spain; and (4) Dpto. de Biología, Universidad de Padova, 35121 Padova, Italy. E-mail:ferpliego@uma.es

Softening of strawberry fruit during ripening and post-harvest is one of the main causes of quality loss in this perishable fruity. The most noticeable change taking place during ripening is the dissapearance of the middle lamella of the walls of the cortical parenchyma cells, releasing pectins, although cellulose and hemicelullose degradation can also contribute. The underlying biochemical basis for this process is largely unknown, although induction of several wall hydrolytic enzymes, pectate lyase and cellulase, have been demonstrated. To ascertain the role of both genes in the control of fruit softening, we obtained transgenic plants of strawberry, cv. Chandler, with the antisense sequence of pectate lyase (ANTIPEL) or celulase (ANTICEL) under the control of the 35S promoter. At this moment, experiments are in progress to get transgenic plants with the double construct pectate lyase-celulase. Fruits of ANTIPEL plants were firmer than those of control plants, non transformed or transformed with the binary vector pGUSINT. This firmness has been kept thoughout two fruiting seasons in vegetatively propagated progenies. No other fruit traits were significantly modified by transformation, however yield was reduced in most transgenic plants. In relation to ANTICEL plants, no significant changes in fruit firmness or other agronomical traits were detected during the first ex vitro growing season, although a reduction in yield was also observed. Interestingly, some plants transformed with pGUSINT also showed a decrease in production in relation to control, non transformed plants. These results show that while pectate lyase seems to be involved in strawberry fruit softening, cellulase does not play a major role. The decreased yields observed in most transgenic lines could be the result of transformation itself linked to the adventive regeneration process used.

P-1270

Cloning and Expression of the Cranberry Gene Encoding Dihydroflavonol-4-Reductase. JAMES POLASHOCK[1], Raymond Sullivan[1], Nicholi Vorsa[1], and Robert Griesbach[2]. [1]Department of Plant Science, Rutgers University, Chatsworth, NJ 08019, and [2]U.S. Department of Agriculture, Beltsville, MD 20705-2350. E-mail: Polashock@aesop.rutgers.edu

Cranberry (*Vaccinium macrocarpon* Ait.) contains many compounds with important implications for human health. Cranberry A-type proanthocyanidins, or condensed tannins, may prevent urinary tract infections and the flavonol quercetin is both an androgen and oestrogen receptor. Anthocyanin pigments found in the flowers, fruits and other parts of higher plants provide an important dietary source of antioxidants. Proanthocyanidins, flavonols, and anthocyanins are products of the flavonoid biosynthetic pathway. Both qualitative and quantitative manipulation of flavonoids would enable development of optimal flavonoid profiles for human health. One way to manipulate their production is through cloning and over-expression in transgenic plants of genes encoding key enzymes in the pathway. The gene encoding dihydroflavonol-4-reductase (DFR) was cloned from the cranberry cultivar 'Early Black'. Its sequence similarity was highest to *Camellia sinensis* (tea). This is not surprising since *C. sinensis* is taxonomically the most closely related plant in the current database. The gene was modified for over-expression in plants by fusion to the CaMV 35s promoter. Flowers of transgenic tobacco plants expressing the gene were much darker pink than the controls. The specific anthocyanins produced in the tobacco flower were consistent with predictions regarding the cranberry gene substrate specificity. Expression in tobacco confirmed the identity and function of the clone as well as the potential for increasing anthocyanin accumulation by over-expression of DFR.

P-1271

Selectable Marker Systems for Genetic Engineering of Grapevine. G. M. REUSTLE, M. Wallbraun, M. Zwiebel, R. Wolf, T. Manthey, G. Krczal. Centrum Gruene Gentechnik, SLFA Neustadt, D-67435 Neustadt/W. E-mail: greustle.slfa-nw@agrarinfo.rlp.de

Genetic engineering is a new approach to create pathogen resistance in elite grapevine rootstocks and varieties. Kanamycin was found to be efficient in selecting and regenerating genetic modified grapevine plants. However, public concerns and safty discussion about the release of genetic engineered plants in the environment demand marker free transgenic plants or at least the use of selectable markers others than antibiotics. Efficiency of selectable marker systems to regenerate genetic modified grapevines (*Vitis* spp.) were investigated: i) Phosphomannose-Isomerase (PMI), ii) Phosphinothricin Acetyltransferase (PAT). Agrobacterium mediated transformation of embryogenic tissue of Seyval blanc and different rootstocks using a GUS-Intron cassette combined with the different selectable marker systems were carried out. With the PMI system the number of GUS expressing embryogenic explants increased with Mannose concentrations of 10 to 15 mg/L and without simultaneous supply of sucrose. With continued selection, independent from the Mannose concentration, somatic embryos started the conversion process. Non of the regenerating embryos were transgenic, and very few showed GUS expressing spots. Following transformation of the PAT-GUS-Intron construct, explants exposed to phosphinothricin (PPT) turned deeply brown (Seyval blanc from 15 to 20 mg/L, rootstocks from 2.5 to 5 mg/L) within the first 4 weeks of selection. However, in some cases these explants generated new somatic embryos and embryogenic tissue, showing GUS positive reaction. After transfer of the arising embryos to PPT free medium, transgenic grapevine plants could be regenerated.

P-1272

DNA Typing of *Musa* spp. Collected in Sri Lanka by Simple Sequence Repeats. W. L. G. SAMARASINGHE[1], A. M. Nahfees[2], A. L. T. Perera[2], I. P. Wickramasinghe[2], and D. Kaemmer[3]. [1]Plant Genetic Resource Center, Sri Lanka; [2]Dept. Agric. Biology, Faculty of Agriculture, University of Peradeniya, Sri Lanka; and [3]Centro de Investigacion Cientifica de Yucatan, A.C., Merida, Mexico. E-mail:gaminisam@yahoo.com, pgrc@slt.lk

Biological diversity of *Musa* is rich in Sri Lanka. This diversity may carry resistance to major pests and diseases. Therefore, it is a great asset for banana improvement. Morphological characterization is being done at the Plant Genetic Resources Center in Sri Lanka, but there were no molecular data available yet. Molecular characterization allows to identify duplicates, determine phylogenetic relationships and estimate genetic diversity. In order to identify cultivars and to determine phylogenetic relationship, seven Simple Sequence Repeat (SSR) primer pairs were used. DNA was extracted from 14 AA, AAA, AAB, ABB cultivars and one AA wild species (*Musa acuminata* Colla). PCR products were detected in urea PAGE sequencing gels followed by silver staining. A total of 35 alleles were detected in all seven polymorphic loci. Even though diploid and triploid 'A' genomes exhibited high polymorphism, the presence of 'A' genome can be identified in interspecific hybrid cultivars. It shows evidence that 'A' genome in the genotypes may have come from different sources or it may have evolved from long term mutation. However, 'B' genome did not exhibit much variation. Multivariate analysis of molecular data allowed clustering into four major groups. Cultivars within each of three clusters were in agreement with the morphological classification, whereas the other cluster consisted of several genomic groups. Wild diploid *M. acuminata* could be one of the parents of five cultivars (Galanamalu, Ratahondarawale, Muwanethikesel and Mondon). Diploid cultivar Navari (AA) showed higher molecular differences in contrast to all other cultivars. This evidence supports the veiw that Navari is a probable endemic cultivar to Sri Lanka.

P-1273

Developing Transgenic Tomato Lines Carrying Plant Defense Genes. SCOTT C. SCHAEFER, Sergei F. Krasnyanski, Tae-Seok Ko, and Schuyler S. Korban. Department of Natural Resources and Environmental Sciences, 310 ERML, University of Illinois, Urbana, IL. 61801. Email: scschaef@uiuc.edu

Plants exhibit a wide array of defense mechanisms, both static and dynamic. Included in these defense responses are the pathogenesis-related (PR) proteins, which have been shown to exhibit antifungal activity. In this study, maize chitinase and maize glucanase genes were separately transferred into 8-d-old cotyledons of tomato (*Lycopersicon esculentum*cv. Sweet Chelsea) via *Agrobacterium tumefaciens*. Each gene was driven by a CaMV 35S promoter, and the construct also contained a *uidA* reporter gene, and an *nptII* gene for kanamycin selection. Leaf tissue from T_0 plants has tested positive for B-glucuronidase (GUS) enzyme activity. These tomato lines were also confirmed to carry the transgene using Southern blot analysis. The level of protein has been determined following western blot analysis. The transgene activity will be compared to plant reaction upon inoculations with *Phytophthora infestans* (tomato late blight), *Alternaria solani* (tomato early blight), and *Septoria lycopersici* (septoria leaf spot). The level of disease resistance in these various tomato lines will then be assessed.

P-1274

Cloning and Partially Characterizing 5S Ribosomal RNA Gene Repeat Units from Two Carica Species Using PCR. MAHIPAL SINGH and Anand K. Yadav. Agricultural Research Station, Fort Valley State University, 1005 State University Drive, Fort Valley, GA 31030–4313. Email: singhm@mail.fvsu.edu

Nuclear encoded 5S rRNA genes (5S rDNA) occur in high numbers as tandemly arranged repeats with their sizes varying from about 200 to 900 bps in most of the eukaryotes. They have a highly conserved coding region and a divergent non-transcribed spacer which differs in length, sequence or the copy numbers of the repeat units. The number of 5S rRNA genes occurring in the plant species is frequently much higher than those of 18S, 5.8S, and 25S rRNA genes. Except in the case of *Marchantia polymorpha*, the 5S rRNA genes are usually not linked to those of 18S, 5.8S, and 25S rRNA genes. Literature search indicated that the molecular studies pertaining to the organization of these genes in the cultivated *Carica* species which produce highly nutritious fruits in the warmer climates, have not been reported thus far. Therefore, the purpose of this investigation was to examine the organization of 5S robosomal RNA genes in Carica species. In this study, consensus primers complementary to and based on the sequences of the 3' and 5' ends of the 5S rRNA gene coding regions for plants were designed as forward and reverse primers. Using these two primers 5S ribosomal RNA gene repeat units were PCR amplified from *Carica quercifolia* and *Carica papaya* genomes. As many as six amplified products ranging from 300 bps to 2000 bps were obtained. The intensity of the PCR products on agarose gels decreased from the lower molecular size to the higher molecular size. The size of the amplified products also appeared to be multiples of the lowest molecular weight PCR products. Both of these *Carica* species carry different sizes of amplified products. Various amplified products of different sizes were cloned in the pCR4 TOPO vector and characterized with respect to their size and amplification profiles which showed putative multiple repeats. However, the on-going sequencing of these clones should reveal the nature of the 5S rRNA repeat units.

P-1275

Transformation of Apple with the Stilbensynthase Gene from *Vitis vinifera* L. and the PGIP-gene from *Actinidia deliciosa*. I. SZANKOWSKI*, H. Kiesecker**, J. Schönherr*, and H.-J. Jacobsen**. *Insitute of Vegetable and Fruit Science, Fruit Science Devision, University of Hanover, Am Steinberg 3, D-31157 Sarstedt, Germany. **Department of Molecular Genetics, University of Hanover, Herrenhäuserstr. 2, D-30419 Hannover, Germany. E-mail:IRIS.SZANKOWSKI@STUD.UNI-HANNOVER.DE

Traditional apple breeding is a very slow and lengthy process because of the long juvenile period and the high level of heterozygosity of this perennial fruit tree. Genetic engineering offers the possibility to introduce novel genes into fruit crops like apple to improve existing varieties by changing a key attribute such as disease resistance without changing the varietal identity. One important part in plant responds to pathogen attack is the induction of antimicrobial compounds called phytoalexins. Several plants synthesize the stilbene type phytoalexin resveratrol. This compound is synthesized by the enzyme stilbene synthase. The precursor molecules for the formation of resveratrol, malonyl-CoA and p-coumaroyl are commonly present in the plant. The stilbene synthase gene was transferred into several plant species and let to an increased disease resistance in those plants. Some facts indicate that also cell wall bound polygalacturonase-inhibiting proteins (PGIP) play a role in plant defense. They are capable of inhibiting fungal endopolygalacturonases. Because of their different specifities the heterologous expression harbours the chance to increase plant defense responses.The cultivars Elstar and Holsteiner Cox belong to the most commercially important apple cultivars in Germany. These and many varieties are very susceptible to fungal diseases like apple scab, the most widespread disease in apple orchards worldwide. We have produced one transgenic line of the cultivar Elstar harbouring the stilbensynthase gene and several transgenic lines of the cultivar Holsteiner Cox with the stilbensynthase gene from *Vitis vinifera* L. and the PGIP-Gene from Kiwi (*Actinidia deliciosa*) respectively. All lines contain the selectable marker gene *bar* from *Streptomyces hygroscopicus* which effects the resistance against the herbicide phosphinotricin. Integration of the transgenes were confirmed by southern blot analysis and expression of the genes were positively checked via RT-PCR. Further experiments will focus on the evaluation of the disease resistance level of the transgenic plants in comparison to not transformed control plants. In addition the transgenic character of the vegetativly propagated plants will be further investigated because of the possible transgene dilution in putative chimeric plants. Acknowlegdement: We thank Prof. Dr. Hain (Bayer AG) for providing the *vst*-construct.

P-1276

Magainin and *npt-II* Gene Co-integration in Grapevine Genomic DNA After Particle Co-bombardment. J. R. VIDAL, J. R. Kikkert, P. G. Wallace, and B. I. Reisch. Department of Horticultural Sciences, New York State Agricultural Experiment Station, Cornell University, Geneva, NY 14456. E-mail: bir1@cornell.edu

Magainins and their analogs are short linear peptides with alpha-helical structure reported to have a broad spectrum antimicrobial activity. Embryogenic cell suspensions of 'Chardonnay' (*Vitis vinifera* L.) were co-bombarded with the plasmid pSAN237, which harbors the *npt-II* gene driven by the *Arabidopsis* ubiquitin-11 promoter, and a second plasmid with a magainin-type gene driven by the *Arabidopsis* ubiquitin-3 promoter. Co-transformation was tested with four separate magainin constructs: pSAN167 (natural magainin-2, Mag-II), pSAN168 (synthetic magainin, MSI-99), pSAN315 (natural magainin, PGL) and pSAN319 (magainin-2 + PGL). After co-bombardment, putative transgenic embryos were selected in kanamycin- containing half-strength Murashige and Skoog medium and then transferred to kanamycin-free Woody Plant Medium for rooting and shoot development. In total, 78, 91, 128 and 107 embryos were selected within the first six months after co-bombardment with pSAN167/237, pSAN168/237, pSAN315/237 and pSAN319/237, respectively. On average, 51% of selected embryos developed into normal plants in vitro within the first year. PCR amplifications with specific primers for the *npt-II* gene and the antimicrobial peptide genes are underway to detect the integration of both genes into genomic DNA. The percentage of co-integration is presently being studied. Embryo development on medium containing kanamycin and PCR amplification of the *npt-II* gene provide good evidence that the *Arabidopsis* ubiquitin-11 promoter is functional in Vitis.

P-1277

RAPD-based Genetic Linkage Maps of Yellow Passion Fruit (*Passiflora edulis*). M. L. C. VIEIRA, M. S. Carneiro, L. E. A. Camargo, A. S. G. Coelho, R. Vencovsky, R. P. Leite, Jr., and N. M. C. Stenzel. Universidade de São Paulo, Escola Superior de Agricultura "Luiz de Queiroz", Departamento de Genética, Piracicaba, 13400–970, Brazil. E-mail: MLCVIEIR@CARPA.CIAGRI.USP.BR

A single cross between two clones of passion fruit (*Passiflora edulis*, 2n=18) was selected for genetic mapping. The mapping population was composed of 90 F_1 plants derived from 'IAPAR 123' (female parent) *x* 'IAPAR 06' (male parent). A total of 380 RAPD primers were analyzed according to two-way pseudo testcross mapping design. The linkage analysis was performed using Mapmaker with LOD 4.0 and max $\theta = 0.30$. Map distances were estimated using the Kosambi's mapping function. Linkage maps were constructed with 269 loci (2.38 markers per primer) of which 255 segregated in a 1:1 ratio, corresponding to a heterozygous state in one parent and null in the other. The linkage map for 'IAPAR123' consisted of 135 markers. A total of nine linkage groups were assembled covering 727.7 cM, with an average distance of 11.20 cM between framework loci. The sizes of the linkage groups ranged from 56 to 144.6 cM. The linkage map for 'IAPAR 06' consisted of 96 markers, covering 783.5 cM. The average distance between framework loci was 12.2 cM. The length of the nine linkage groups ranged from 20.6 to 144.2 cM. On average, both maps provided 61% genome coverage. Twenty-four loci (8.9%) remained unlinked. Among many applications, these maps are a starting point for identifying quantitative trait loci for resistance to the main bacterial disease that affects passion fruit orchards in Brazil, which is caused by *Xanthomonas campestris* pv. *passiflorae*, as parental genotypes respond diversely to bacterial inoculation.

P-1278

Direct Gene Transfer to Passion Fruit (*Passiflora edulis*) with the Atacin A Gene. M. L. C. VIEIRA, E. K. Takahashi, M. C. Falco, L. G. Vieira, and L. F. P. Pereira. Universidade de São Paulo, ESALQ, Depto. de Genética, Piracicaba, Brazil. E-mail: mlcvieir@carpa.ciagri.usp.br

The first objective of the present work was to optimize a regeneration system to be used in direct gene transfer to *Passiflora edulis* plants. Microparticle bombardment was assayed in order to transfer the *atacin a* gene, driven by the 35S–35S promoter, to plants of an elite cultivar of passion fruit. The final idea is to produce transgenic plants resistant to *Xanthomonas campestris* pv. *passiflorae*. A protocol was established from nodal cuttings (2 mm) and morphogenic calli were produced. Scanning microscopy and histological analysis confirmed the course of the regeneration as organogenesis. Shoot regeneration occur in Phytagel (0.18%) solidified MS medium supplemented with BAP (0,5 mg/L). After 18 d of culture the calli surfaces were totally covered by buds. Shoots were excised after 40 d and transferred to 1/2 MS where rooting occurred. Transient gene (*uid/A*) expression was effective in the following condition: 1200 psi of helium pressure and 9.5 cm microprojectile flight distance. Transformation frequencies of 5.17% were obtained. Transgene insertion was confirmed by PCR for both GUS and *atacin* gene.
Financial support: CNPq.

P-1279

Abstract has been withdrawn

P-1280

Non-cryogenic, Long-term Preservation of *In Vitro* Multiple Bud Clusters of Papaya. C. C. Lai[1], S. D. Yeh[2], and J. S. YANG[1]. [1]Department of Botany and [2]Department of Plant Pathology, National Chung-Hsing University, Taichung, Taiwan, Republic of China. Email: jsyang@dragon.nchu.edu.tw

In vitro culture of papaya (*Carica papaya* L. cv. Tainung No. 2) multiple bud clusters was long-term preservated under non-cryogenic conditions. Minimal conditions under moderate growth temperature was achieved by the combination of three preservation conditions; carbon source (3% sucrose or 1% fructose), growth regulator (ordinary or free of growth regulators), light intensity (6, 12, 26, or 53 $\mu Em^{-2}s^{-1}$). After 12-month preservation, the explants preservated on sucrose medium had died, while most of those on fructose medium can be recovered. The condition at 1% fructose without growth regulators under 12 $\mu Em^{-2}s^{-1}$ was available for one year preservation and recovered with 4 shoots per flask. The factor contributions were analyzed that growth regulator (about 50% contribution) was the great factor before month 3, while after 6-month preservation the carbon source factor was the primary factor (up to 75% contribution). In order to predict the regrowth shoot number, polynomial regressions were performed on the cumulative data of the regrowth shoot number and the 2,3,5-triphenyltetrazolium chloride (TTC) viability. For the available one year preservation, the adequate TTC viability of multiple bud clusters at week 3 was 0.8~1.2. These results indicated that the carbon source, 1% fructose, is available as the primary factor for long-term preservation of papaya multiple bud clusters under moderate growth temperature and this process can be predicted by TTC analysis at week 3.

P-1281

Partial Purification of Acid Phosphatases from Tomato and Garlic Seedlings. BEGÜM YENİGÜN, Nuran Deveci, and Yüksel Güvenilir. İstanbul Technical University Department of Chemical Engineering, 80626 Maslak, İstanbul, TURKEY.

Acid phosphatase is a phosphatase of low specificity and the natural substrates are unknown. The enzyme occurs in a variety of tissues, e.g., liver, spleen, erythrocyte, and prostate. The highest concentration is present in the prostate and the detection of prostatic carcinoma is the main purpose of clinical essays of this enzyme. Approximately one-third of the normal circulating acid phosphatase in adult males is derived from the prostate. Acid phosphatase or ortophosphoric monoester phosphohydrolase are a group of hydrolyses that catalyze the hydrolysis of a large number of orthophosphoric monoester compounds within the acid range of pH. The purpose of this study was the partial isolation and purification of acid phosphates from two of the plant sources, tomato seedlings and garlic seedlings, and to determine some characteristics. Acid phosphatase was isolated and purified from garlic and tomato seedlings by a stream line method without the use of proteolitic and lipolytic enzymes, buthanol, or other organic solvents. Grown garlic and tomato seedlings of about 10 cm height were homogenizated with 0.1 M acetate buffer containing 0.1 M NaCl and 0.1% Triton X-100. While three-step fractionation of the proteins with ammonium sulfate was applied to filtrated supernatant of garlic seedlings, two-step ammonium sulfate fractionation was applied to filtrated supernatant of tomato seedlings. Garlic seedling acid phosphatase was purified with DEAE-cellulose ion exchange chromatography. Tomato seedling acid phosphatase is applied to DEAE-Sepharose ion exchange chromatography after dialysis step. While garlic seedling acid phosphatase was purified 40 fold from the starting material, purification fold for tomato seedling acid phosphatase was 3. The specific activity of the garlic acid phosphatase is 168 Unite/mg of protein and the specific activity or tomato seedling acid phosphatase is 13 Unite/mg of protein after purification. A variety of stability and activity profiles are determined for both garlic and tomato seedling acid phosphatase. These include optimum pH, optimum temperature, pH stability, and temperature stability. For garlic seedling acid phosphatase, the pH optimum is 5.70 and the optimum temperature is 50° C. The enzyme is stable at 4–10 pH and 40–60° C. For tomato seedling acid phosphatase, optimum pH is 5.60, optimum temperature is 30° C. Enzyme is stable between 0–50° C and 5.0–6.8 pH.

P-1282

Improvement of Wheat Baking Quality by Gene Engineering. XIAODONG ZHANG, Xvqing Chen, Fengping Yang, and Rongqi Liang. Beijing Agro-Biotechnology Research Center, Beijing Academy of Agricultural & Forestry Science, Beijing 100089 China. E-mail: zhxd2000@hotmail.com

The unique baking quality of wheat (*Triticum aestivum L.*) flour is highly associated with the number and composition of high molecular weight glutenin subunits (HMW-GS), which are very important in determining gluten and dough elasticity. In previous studies, recombinant plasmids, pBPC30 (11kb) and pBPC31 (12kb), which both contain HMW-GS 1Dx5 and 1Dx10 genes, and bar gene as selection marker, were constructed. An efficient procedure for wheat plant regeneration from immature inflorescence, anthers, immature embryos and embryogenic calluses was established. More than 500 herbicide resistant transgenic plants were produced from explants of common wheat cultivars as immature inflorescence, anthers, immature embryos and embryogenic calluses, following biolistic microprojectile bombardment (PDS-1000/He) and selection on medium with bialaphos or phosphinothricin (PPT). All of transgenic lines and their progenies (T_5 and T_6 up to now) were morphologically normal and fertile. Transgenic plant lines identified by dot blotting and Southern blotting showed that bar, 1Dx5 and 1Dy10 genes have been integrated into genome of some transgenic lines. Analysis of seed proteins by SDS-PAGE showed that HMW-GS 1Dx5 and 1Dy10 genes were well expressed in offspring seed of transgenic lines. Substitution between HMW-GS, for example, Subunit 2 replaced by Subunit 5, Subunit 12 replaced by Subunit 10, and random insertion are the main way of foreign gene integration. There are several types of HMW-GS composition found in T4 seed of transgenic line TG3–74: 2+5+7+10+12, 2+5+7+*+10+12, 5+7+8+10, 5+7+10, 2+7+10+12, etc. With quality property assay on seed proteins of 102 T_5 plants, the Sedimentation Value by Zeleny method of 5 transgenic lines (44.2–49.0) was similar to bread wheat Cheyenne (48.0). In 25 transgenic lines from Jinghua No.1, the Sedimentation Value of 9 transgenic lines, ranging from 29.4 to 46.1, was remarkably higher than that of the wild type (26.9), increasing 9.3–74.3%. The total seed protein content, ranging from 12.8% to 15.1%, was also higher than that of wild type (11.6), increasing about 10.3–30.2%. And the protein content of flour ranges from 10.7% to14.4%, increasing 26.3–48.5% compared with that of wild type (9.7%). In 26 transgenic lines from Jingdong No.6, the Sedimentation Value of 10 transgenic lines, ranging from 37.1 to 49, increasing 20.9–59.6% with the wild type (30.7). Analysis of dough rheological properties by BRABENDER farinograph showed that the dough stable time of 5 transgenic lines range from 16 minutes to 30 minutes, however that of wild types were only 3 or 4 minutes. Our research showed that the improvement of bread-making quality could be achieved by engineering novel HMW-GS genes. Key Words: Wheat (*Triticum aestivum L.*), HMW-GS, Transgenic, Baking Quality.

P-1283

Flower Mutants of Dahlia (*Dahlia pinnata* Cav.) Induced by Heavy-ion Beams. T. ABE[1], M. Hamatani[2], Y. Iitsuka[2], N. Fukunishi[1], K. Miyoshi[3], M. Yamamoto[4], S. Yoshida[1]. [1]Plant Functions Lab., RIKEN, Wako, Saitama 351–0198, JAPAN; [2]Hiroshima City Agriculture and Forestry Promotion Center; [3]Faculty of Bioresource Sciences, Akita Prefectural University, and [4]The Hiroshima Botanical Garden. E-mail: tomoabe@riken.go.jp

Dahlia is one of the important crop for cut flower industry during winter time in Horoshima City. However, the number of the varieties, which are adapted for winter production, is quite limited. Radiation has been widely adopted in order to induce artificially mutations of various plant species as an easy and effective way. The irradiation of heavy-ion beams as a new method for the induction of dahlia mutations were more stable and in wider spectrum of variation than ones with gamma rays. Irradiation treatments were conducted on the shoots (ca.1 cm in length) of pink flower dahlia cv. 'Miharu', which were grown in the modified MS medium, in dose range of 5–20Gy for N-ion. Plants treated with 10Gy grew vigorously in vitro as well as in the experimental field, and showed highest frequency of mutant induction. The results under the cultivation of experimental field are as follows; with the increase in exposure dose, 1) decrease in frequencies of anthesis, 2) increase in the variation of flower diameter, 3) increase in the variation of flower colors, 4) increase of the malformation of flower. As for floral diameter, those of 3–12 cm have been observed. The mutants, such as petals in darker or paler colors or with white tip petal were observed observed in the present experiment and these were observed in our previous experiment with gamma-ray irradiation. Mutants with darker floral colors were more commonly observed by the irradiation of N-ion than gamma-rays. Frequency of mutants with darker flower colors is higher than paler ones. The analysis for the color of the flowers with chroma meter, showed that the mutations of floral colors after 5Gy and 10Gy were not continuous variation but marked two peaks. More than 10 clones with flowers of pink and white tip petal, with pale colors, and with darker colors are selected as promising strains for marketability.

P-1284

The Use of the GAI and CO Genes to Create Novel Ornamental Plants. CHARLEEN BAKER, Hong Zhang, Gerald Hall, David Scrocki, Angelica Medina, and Michael S. Dobres. NovaFlora Inc, 3401 Market Street, Suite 350, Philadelphia, PA 19104. E-mail: cbaker@novaflora.com

Novelty and uniqueness are key factors dictating the value of ornamental plants. In addition, grower traits that lower production costs and reduce losses are also of significant value. The GAI gene (Gibberellic Acid Insensitive) was cloned from Arabidopsis thaliana (thale cress) by the research group of Dr. Nicholas Harberd at the John Innes Center, UK. GAI is involved the perception of Gibberellic Acid (GA). GA is a natural plant growth-regulator that promotes stem elongation. By expression of specific GAI gene variants NovaFlora can block the ability of the plant to perceive GA levels and thereby effectively control the height and stature of a plant. NovaFlora is using GAI to develop a range of novel dwarf ornamental flowers, shrubs and trees. The research group of Dr. George Coupland has shown that overexpression of the CO gene can cause early flowering in the model plant Arabidopsis, and that overexpression of CO can override the plant's requirement for a specific day-length. NovaFlora has used the CO gene to induce early flowering in petunia under short-day conditions that delay flowering wild-type plants. This demonstrates that CO works in heterologous species, as it does in Arabidopsis, to shorten flowering time and reduce a plants 'seasonal' requirement for flowering. NovaFlora is using CO to develop a range of ornamental plants with reduced daylength, and hence reduced supplemental light requirements. NovaFlora has entered into an exclusive license agreement with Plant Biosciences Limited and the John Innes Center, UK, to commercialize the GAI and CO genes in ornamental plants.

P-1285

Factors Affecting Transient Expression of the *gus*A Reporter Transgene in *Cymbidium* Protocorm-like Bodies. MURRAY R. BOASE, T. A. Lill, M. J. Bendall and D. B. Horgan. New Zealand Institute for Crop & Food Research Limited, Private Bag 11–600, Palmerston North, New Zealand. E-mail: boasem@crop.cri.nz

Cymbidium orchids are the leading cut flower export crop for New Zealand. To genetically engineer the flavonoid biochemical pathway in cymbidiums, a reliable cultivar-independent genetic transformation system is required. Experiments with a modified particle inflow gun were conducted to optimise parameters involved in the transformation of *Cymbidium* protocorm-like bodies (plbs). The percentage of plbs with blue foci, at 3 days post bombardment, varied with cultivar: More plbs of Rose Armstrong "Sun Ray" displayed blue foci than Vala which in turn had more than Rose Armstrong "First Choice". The effect of shooting distance varied with cultivar: For Rose Armstrong "First Choice" increasing the shooting distance from 11 to 13 cm significantly reduced the average number of blue foci per plb but for Vala this effect was not statistically significant. Solenoid opening times (SOTs) were varied from 10 to 50 millisecs in 10 millisec intervals, when shooting cultivar "Fuss Fantasy": 40 and 50 millisec SOTs gave significantly higher averages of blue foci per plb than 10, 20 or 30 millisec SOTs. Gold precipitation events were found to be very significant: One precipitation event gave more than twice the average number of blue foci per plb of Rose Armstrong "Sun Ray" than two other events. In a GUS time course experiment with this cultivar, the percentage of plbs with blue foci was constant at 7, 14 and 21 days but fell at 28 and 42 days, along with the average numbers of blue foci per plb. GUS positive plbs of cv. Fuss Fantasy have been obtained after 9 months of geneticin selection and transgenic shoots are regenerating.

P-1286

Genetic and Epigenetic Aspects of Somaclonal variation: Azalea Flower Colour Bud Sports, a Case Study. S. DE SCHEPPER, P. Debergh, E. Van Bockstaele, M. De Loose. Department of Plant Production, University Gent, Coupure links, 653, B-9000 Gent, BELGIUM. Email: sandra.deschepper@rug.ac.be

Bud sporting is the consequence of sudden variations in the gene expression of somatic cells, leading to the occurrence of phenotypically altered shoots on plants. This phenomenon is characteristic for vegetatively propagated plant species. In azalea, the frequent occurrence of flower colour sports is appreciated as a valuable additional source of variation; more than half of the commercial assortment consists of flower colour bud sports. However, when clonal uniformity is desired for registration and propagation, it is perceived as a hindrance. Insight into the molecular mechanism(s) underlying the induction event might improve breeding strategies, towards the appropriate choice of cultivars in hybridization experiments. Azalea flower colour sports are characterized by different types of variegation. According to the colorization type, the origin of the induction event is situated more likely at the genetic or epigenetic level. As genetic mechanisms, we have considered mutations, transposable elements, chimerism, and polyploidy. At the epigenetic level, we investigated gene silencing and evaluated the methylation state of sports. An AFLP-fingerprint excluded the possibility of DNA polymorphisms between sports. Transposon-specific PCR revealed the presence of *hAT*-like transposons in azalea. Microscopy and flow cytometry excluded the periclinal chimeric nature of picotee sports and indicated a topographic determination of colour and ploidy. Northern and RT-PCR analysis proved the epigenetic regulation of the *chalcone synthase* flavonoid gene. Global methylation analysis revealed methylation polymorphisms between the clonally-related sports.

P-1287

Induction of Sterility in Transgenic Plants by Melon Ethylene Receptor Genes *Cm-ERS1* and *Cm-ETR1*. H. EZURA[1], K. Yuhashi[2], K. Takada[1], H. Kamada[1]. 1. Gene Research Center, University of Tsukuba, Tsukuba 305–8572, Ibaraki, Japan. 2. JST and Plant Biotechnology Institute, Ibaraki Agricultural Center, Iwama, Nishi-ibaraki 319–0292, Japan. E-mail: ezura@gene.tsukuba.ac.jp

We have isolated melon ethylene receptor genes, *Cm-ERS1* and *Cm-ETR1* (Sato-Nara et al., 1999), and produced transgenic plants with these genes. Transgenic tobacco plants with *Cm-ERS1* and the mutant form *H70A* which protein lacks an ethylene binding activity showed reduced-sensitivity to ethylene and delayed flower senescence. The transgenic tobaccos also showed less pollen production and restricted pollination due to their modified flower architecture (long-styled flowers), and these changes resulted in less or no seed production in the transgenic plants. Crossing and segregation analyses demonstrated the correlation between these reproductive characteristics and transgenes. In addition, *Cm-ERS1* and *H70A* were introduced to other kinds of plants including tomato, lettuce and *Lotus japonicus*. Some lines of these transgenic plants also showed less or no seed production. We also produced transgenic tobacco plants with a mutant form of *Cm-ETR1*, *H69A* that has a mutation in ethylene binding residue and should confer insensitivity to transgenic plants. The transgenic tobacco plants showed similar and severe phenotypes to those with *Cm-ERS1* and *H70A*, and did not set seeds by open pollination. These results suggest that ethylene receptor gene is an alternative tool for conferring sterility to transgenic plants.

P-1288

Development of *Lilium longiflorum* Cell Cultures of High Competence for Transformation by Particle Bombardment and of High Embryogenic Capacity A. LIPSKY[1], A. Cohen[1], R. Barg[2], S. Shabtai[2], Y. Salts[2], V. Gaba[3], K. Kamo[4], A. Gera[3], and A. Watad[1]. [1]Departments of Ornamental Horticulture, [2]Plant Genetics, and [3]Virology, ARO The Volcani Center, Bet Dagan 50250, Israel, and [4]Florist and Nursery Plants Research Unit, USDA, Beltsville Agricultural Research Center, Beltsville, MD, 20705. E-mail: abedg@netvision.net.il

Lilium species (Liliaceae) are a significant floriculture commodity and one of the three major bulb crops in the commercial market. Lilies are monocotyledonous plants that have been generally recalcitrant to molecular genetic manipulation because of restrictions on the utilization of transformation technologies that are routinely applied to dicotyledonous plants. Previously we have found problems of unstable transformation with *Lilium*. To produce stable transformants we established highly morphogenic cultures in liquid medium from calli derived from segments of sterile bulblet scales of *L. longiflorum* Thunb. cv. Snow Queen. Callus cultures from bulblet scales had relatively low morphogenic potential after 6 months subculture. Competence for genetic transformation of three- or ten-month old cultures maintained in a liquid medium in shake flasks in darkness was investigated by microprojectile bombardment, using a Finer-type bombardment apparatus. Gold particles were coated with plasmid pUBQ3genGUS, containing the uidA reporter gene under the control of promoter UBQ3. In each experiment three bombardments were performed of each of two of Petri dishes with explants, and 10,000 to 15,000 GUS transient expression foci were found. The liquid-grown tissue cultures have a level of competence for transformation about 50–70 times greater than that of solid-grown callus cultures. Regeneration of 81.6 ± 3.7 normal plantlets per g FW resulted from such tissue cultures in a liquid medium in shake flasks in the light. There was a high level of somatic embryoid production during early regeneration stages. Growth and regeneration of the cultures in liquid medium were blocked by a low concentration of the antibiotic kanamycin. Kanamycin was more effective for selection than the herbicide bialaphos. The effectiveness of the method for genetic transformation was confirmed by obtaining tens of stable transgenic plants of *L. longiflorum* Thunb. cv. Snow Queen. Most recently, we have produced over a hundred individual transgenic plants using a plasmid based on pCAMBIA 2301, containing a *rol B* gene under the control of the *Lat 52* promoter, to prevent pollen development. The GUS gene is expressed strongly throughout these plants. A molecular and phenotypic analysis of these plants is currently being performed.

P-1289

Development of *Ornithogalum dubium* Cell Cultures of High Competence for Transformation by Particle Bombardment and of High Embryogenic Capacity A. LIPSKY[1], A. Cohen[1], V. Gaba[2], A. Ion[1], D. Sandler-Ziv[1], A. Gera[2], and A. Watad[1]. [1]Department of Ornamental Horticulture, and [2]Department of Virology, ARO The Volcani Center, Bet Dagan 50250, Israel. E-mail: vhacohen@agri.gov.il

Ornithogalum species are a high value commercial floriculture bulb crop. *Ornithogalum* is a monocotyledonous plant that has been recalcitrant to molecular genetic manipulation because of limitations that restrict utilization of transformation technologies that are routinely applied to dicotyledonous plants. For optimization of genetic transformation of *O. dubium* cv. ATD we tested the *in vitro* regeneration potential of leaf segment explants and cultures in liquid shake flasks, and subsequently transient expression of the GUS gene. Previously good shoot regeneration was observed from the tips of leaf segments. The segments held vertically were bombarded three times per side using a Finer-type of bombardment apparatus and gold particles coated with plasmid pUBQ3genGUS, containing the uidA reporter gene under the control of promoter UBQ3. In an experiment with 111 leaves segments only 202 GUS transient expression foci were found. Calli derived from the basal part of axenic plantlets were used to establish cultures of high morphogenic potential in liquid medium. After 8 weeks growing on agar-solidified medium 206 (MS salts and vitamins, 3% sucrose, 2 mg/l BA and 0.1 mg/l NAA) callus cultures were transferred to liquid medium 101 (MS salts and vitamins, 3% sucrose, 0.1 mg/l BA and 1 mg/l NAA). Competence for genetic transformation of the cultures in the liquid media after six subcultures (9 weeks) in shake flasks in darkness was investigated by microprojectile bombardment. In each experiment three bombardments were performed of each of two of Petri dishes with the different cultures. For the cultures grown in medium101 2,300 GUS foci were observed, and 6,500 GUS foci were found in material cultured in medium 206. The liquid-grown cultures have a level of competence for GUS transient expression in medium 101 about 11 times greater than that of the leaf segments, and in medium 206 almost 33 times greater. Morphologically, liquid-grown tissue cultures in medium 206 have had more compact globules (diameter 2 – 4 mm) than in medium 101 (diameter 2 – 8 mm). Regeneration of these tissues in the same liquid medium (MS with 3% sucrose) in shake flasks in the light was different. Regeneration of cultures grown in medium 101 it was organogenic, but from medium 206 somatic embryoids were produced. Growth and regeneration of the established cultures in liquid medium were blocked by a low concentration of the antibiotic kanamycin, without necrosis to a high concentration. Kanamycin was more effective than the herbicide bialaphos. Efficiency of the liquid-grown tissue cultures in the medium 206, with negative selection and plant regeneration will be confirmed by soon by genetic transformation experiments.

P-1290

Genetic Engineering of Chemically Reversible Male Sterility by Expressing CKX1 in Transgenic Maize. SHIHSHIEH HUANG, R. Eric Cerny, and Youlin Qi. Mystic Research/Monsanto Company, Mystic, CT 06355. E-mail: shihshieh.huang@na1.monsanto.com

Many crop plants have a greater yield potential as hybrids. However, the difficulty of obtaining a pure source of out-crossed seed in crops which usually contain functional male and female organs on the same flower or plant, has limited the use of hybrids. Biotechnology provides new possibilities for improving current hybrid seed production systems. We have genetically engineered a reversible male sterility system in transgenic corn by expressing a gene, *CKX1*, involved in oxidative cytokinin degradation, in male tissues. Male sterility of these transgenic plants is caused by the accumulation of CKX1 and the fertility can be restored by application of a synthetic cytokinin, Kinetin, and a cytokinin oxidase inhibitor, thidiazuron (TDZ). This technology creates a new method for generating and maintaining male sterility in plants, which could not only reduce the expense of seed production for existing hybrid crops but also make it possible to produce hybrid varieties of traditionally non-hybrid crops.

P-1291

gai Induced Male Sterility Is Reversible by Kinetin. SHIHSHIEH HUANG, Youlin Qi, R. Eric Cerny, Deepti Bhat, Carrie M. Aydt, and Kathleen P. Malloy. Mystic Research/Monsanto Company, Mystic, CT 06355. E-mail: shihshieh.huang@na1.monsanto.com

Earlier experimental approaches on a number of male sterile systems and normal plants have shown that gibberellins (GAs) are positively involved in the male development of flowering plants, but they also can cause male sterility and promote the female development in a few instances. By using molecular approaches, we expressed *gai*, a GA-insensitve mutant gene, specifically in anthers and pollen of transgenic tobacco and Arabidopsis plants and, the anther and pollen development of the transgenic plants were aborted. Thus, it confirms that GAs are essential for the male development in these two species and also demonstrates a new approach for studying GAs' involvement in the male development. Furthermore, the male sterile phenotype exhibited by the transgenic plants is reversible by exogenous application of kinetin. These results support the hypothesis that the male development is controlled by the concert interaction of more than one phytohormones. This reversible male sterile phenotype could be applied not only for improving hybrid seed production, but also preventing the spread of transgenic pollen to the environment.

P-1292

Transformation of Gladiolus for Resistance to Bean Yellow Mosaic Virus. K. KAMO[1], A. Gera[2], J. Cohen[2], J. Hammond[1]. [1]Floral & Nursery Plants Research Unit, USDA, U.S. National Arboretum, Beltsville, MD 20705. E-mail: kkamo@asrr.arsusda.gov [2]Department of Virology, ARO, The Volcani Center, Bet Dagan, 50250 Israel. E-mail: Abed@netvision.net.il

Many of the floral monocots are perennial crops propagated by bulbs. This method of propagation results in continued transmission and increased incidence of viruses to subsequent plant generations. Viruses cause decreased vigor of the plant, and its symptoms include streaking of the flower petals, chlorosis of the leaves, and distortion of the flower spike resulting in lowering their market value as cutflowers. Transformation of floral bulb crops offers an alternative to conventional breeding as there are no effective genes for virus resistance identified in Gladiolus. Transgenic plants of Gladiolus cultivar Jenny Lee that contained either the bean yellow mosaic virus coat protein or bean yellow mosaic virus coat protein antisense genes were developed by biolistics. Selection for transformants was done using co-transformation with the PAT gene coding for phosphinothricin acetyltransferase under control of the CaMV 35S promoter. Six plants were confirmed to contain the bean yellow mosaic virus coat protein and 11 plants the bean yellow mosaic virus antisense genes by Southern hybridization. The 17 transgenic plant lines were challenged using controlled aphid transmission. One month following transmission of bean yellow mosaic virus by aphids, the transgenic Gladiolus plants were examined by immunoelectron microscopy for presence of the virus. Several transgenic plants lines containing either antiviral gene showed a lower infection rate compared to non-transformed plants.

P-1293

Genetically Engineering Ornamental Plants to Express a More Compact, Free-branching Growth Habit. M. V. KHODAKOVSKAYA, Song Xue*,Yan H. Wu, Yi Li, and Richard J. McAvoy. Department of Plant Science, University of Connecticut, Storrs, CT 06269 and *Horticultural Science Department, University of Florida, Gainesville, FL 32611. E-mail: M_KHOD@YAHOO.COM

A compact, well-branched phenotype is a highly desirable growth form in many ornamental species. However, many species exhibit strong apical dominance or produce long internodes under commercial production conditions. Consequently, plant growth regulators are routinely used to induce a more desirable growth form. Of the chemical plant growth regulators applied during commercial production, approximately 95% (a.i. by weight) is used to reduce internode elongation or to increase lateral branching. Unfortunately, chemical plant growth regulators are expensive, carry a risk of environmental or worker exposure, and often produce undesirable side effects on plant growth and development. In this study, petunia Marco Polo Odyssey was transformed to express reduced auxin activity or increased cytokinin activity in the shoot. Transgenic plants are expected to have shorter internodes and a free- branching phenotype. Using several selected transgenic lines, we are currently characterizing changes in leaf and shoot morphology and the relationship between the levels of transgenic expression and plant form. This technology may be useful for many ornamental plant species.

P-1294

Phylogenetic Relationships Among Commercially Important Ornamental Liriopogons. M. Mcharo, E. Bush, and D. LABONTE. Department of Horticulture, Louisiana State University Agricultural Center, Baton Rouge, LA 70803. E-mail: dlabonte@agctr.lsu.edu

Genetic analyses were conducted on 19 commercially available Liriopogon selections. A total of 184 genetic markers were amplified by the Amplified Fragment Length Polymorphisms (AFLPs) method and the data were analyzed using the Dice coefficientof similarity and unweighted pair-group mean analysis (UPGMA) to determine relatedness. Overall, the molecular analysis showed a homogeneous genetic constitution. Standard varieties of liriope and monkeygrass were selected to show genetic representatives of the entire population. Genetic distances estimated from the molecular marker data showed a low level of diversity between Mondograss and 'Variegated' mondograss and 'Big Blue' liriope and 'Variegated' liriope. Genetic distances estimated from the molecular marker data showed similarity between mondograss and 'Variegated' mondograss. 'Aztec Grass' genetic distances estimated from the molecular marker data showed a closer relationship to Ophiopogon than to Liriope.

P-1295

Large Scale In Vitro Bulblet Production from Immature Embryo Explants of Endemic *Sternbergia candida*. İSKENDER PARMAKSİZ, Semra Mirici,[1] Sebahattin Özcan, Cengiz Sancak, Serkan Uranbey, Bilal Gürbüz, Ahmet Gümüşçü and Neşet Arslan. Department of Field Crops, Faculty of Agriculture, University of Ankara, 06110, Dışkapı, Ankara, Turkey and [1]Department of Biology, Faculty of Science and Arts, University of Kirikkale, Kirikkale-Turkey.

Sternbergia candida is an endangered geophyte endemic to Babadağ in Fethiye province of Turkey and therefore export of its flowerbulbs is forbidden. It grows wild under cedar forests around 1200–1300 meter heights. *S. candida* belongs to *Amaryllidaceae* family and the species in this family contains a series of alkaloids such as tazettin, lycorin, belladin, galanthamin etc. According to their structure, these alkaloids have antimicrobial, anti-viral, anti-leukaemial and immune-stimulant effects. *S. candida* is also used for ornamental purposes. Since *S. candida* is endangered and endemic species, mass propagation of this plant is very important for germplasm conservation and commercial production. Bulbs, corms and other subterranean strorage organs are commonly used *for in vitro* bulblet production in geophytes. In the present study immature embryos were found to be a good alternative source of explants for large scale bulblet production in *S. candida*. Embryo explants were isolated from the immature fruits harvested from native plants and cultured on modified-MS media supplemented with various levels of picloram or 2,4-D. Large scale bulblet production (45–98 bulblets/explants) has been established from immature embryos on media containing 6 mg/l picloram or 2 mg/l 2,4-D after 5–6 months of culture initiation. Regenerated bulblets were were finally transferred to soil. To our knowledge the present study is the first report for *in vitro* bulblet production from immature embryos in geophytes.

P-1296

Benzyl Adenine Utilization: Can It Be Used As A Marker For In Vitro Induction Of Flowering In *Bambusa arundinacea* (Retz.)Willd? MOHINI N. PATHAK [1], R. V. Gadre [2], Rajani S. Nadgauda [1]. [1] Tissue Culture Pilot Plant, [2] Division of Biochemical Engineering, National Chemical Laboratory, Pune-411 008, India. E-mail: rsn@ems.ncl.res.in

In vitro induction of flowering in bamboos has an immense potential in fundamental as well as applied research. We have been working in this area for a decade (Nadgauda et al., 1990) and made a considerable progress in understanding different factors responsible for and exploring the role of growth regulators in *in vitro* induction of flowering in *Bambusa arundinacea*. Cytokinins were thought to play an important role in induction of flowering under *in vitro* conditions. Benzyl adenine (BA) was exclusively essential for induction of flowering in seedling explants when different cytokinins (Adenine hemisulfate, BA, kinetin, 2iP and zeatin) were tested singly and in combination with BA. The absence of BA and/or presence of other cytokinins in the medium resulted in profuse root elongation. When these cytokinins were present in combination with BA, an inverse relationship between root elongation and induction of flowering was noticed. A synergism was observed when 2iP was used in combination with BA, while zeatin in combination with BA showed antagonistic effect on induction of flowering (Joshi & Nadgauda, 1997). The effect of BA on induction of flowering was age specific. We have monitored the residual BA from culture medium through HPLC at different time intervals. Our observations lead us to propose that benzyl adenine utilization can be used as a marker for *in vitro* induction of flowering in this species. Key references: Nadgauda RS, Parasharami VA and Mascarenhas AF (1990) Precocious flowering and seeding behavior in tissue cultured bamboos. Nature 344: 335–336 Joshi MS, Nadgauda RS (1997) Cytokinins and *in vitro* induction of flowering in bamboo: *Bambusa arundinacea* (Retz.) Willd. Current Science 73: 523–526.

P-1297

MADS-box Genes Involved in Inflorescence Development in Asteraceae. O. A. SHULGA*, A. V. Shchennikova*, K. G. Skryabin*, G. C. Angenent. Business Unit Plant Development and Reproduction, Plant Research International, Droevendaalsesteeg 1, Wageningen 6700AA, The Netherlands and *Laboratory of Genetic Engineering, Centre "Bioengineering" RAS, Pr. 60-letiya Oktyabrya 7/1, Moscow, 117312, Russia. E-mail: shulga@biengi.ac.ru

The inflorescence of the Asteraceae, known as a capitulum, possesses a number of features which make it attractive for developmental studies. It contains numerous small individual florets that are arranged in a condensed indeterminate inflorescence, with the outer ones opening first. The most common type of floret is the disk floret, which is usually bisexual and has a tubular corolla. The second type consists of the bilateral ray florets, which are usually pistilate or sterile. Thus at the same time, the capitulum contains various florets at different developmental stages. Sunflower and chrysanthemum belonging to the Asteraceae are very important and widespread oil crop and cutting flower, respectively. Understanding of the processes regulating inflorescence development could be used in genetic engineering of these cultures. Transition of vegetative meristem into floral meristem are controlled by homeotic genes most of which belong to a structurally related group of the MADS-box genes. Their products have been shown to act in combination to determine meristem identity and to specify the fate of floral organ primordia. We have cloned a number of MADS-box genes from sunflower (HAM genes) and chrysanthemum (CDM genes), including homologues of meristem and floral organ identity genes. To investigate the putative functions of these genes we generate transgenic Arabidopsis plants overexpressing HAM/CDM genes. The network of interactions between products encoded by these genes were analyzed in yeast two- and three hybrid systems. According to the presented data two isolated genes, HAM75 and CDM111, are most likely orthologues of the Arabidopsis gene AP1.

P-1298

Genetic Transformation of *Dendrobium* Sonia 17 by Particle Bombardment Using Green Fluorescent Protein (GFP) as Reporter System. C. S. TEE, M. Maziah, [1] C. S. Tan, and A. Puad. Department of Biochemistry and Microbiology, Faculty of Science and Environmental Studies, Universiti Putra Malaysia and [1]Malaysian Agricultural Research and Development Institute (MARDI), Serdang, Malaysia.

Orchid cut flower is one of the major floriculture industries in Malaysia and Dendrobium Sonia 17 is commonly grown for cut flower here. Conventionally, orchid breeders used to cross different orchid hybrids in order to obtain a new hybrid. This traditional method is not promising, unpredictable and time consuming. Genetic engineering of orchid offers a new avenue for orchid breeders to create a new hybrid with more reliable outcomes. In our study, Dendrobium Sonia 17 callus and in vitro inflorescence tips were used as target tissues for bombardment. Two morphologically different callus were identified and used for genetic transformation. Target tissues were co-bombarded using a GFP plasmid, 35S-SGFP-TYG- nos (p35S)and pSM DFR, that carried a hygromycine resistant gene and antisense of dihydroflavonol-4- reductase (DFR) gene. Green fluorescent protein (GFP) was used as a non- destructive reporter system compared to the conventional b-glucorusidase (GUS) assay. The highest GFP transient expression was on the second day of post- bombardment. Physical and biological factors affecting efficiency of transformation were optimised. A combination of GFP and hygromycin selection was used for selection of putative transformed tissues before polymerase chain reaction (PCR) was carried out. The established in vitro flowering system allows earlier assessment of changes in flower colour.

P-1299

Extending Flower Life of Ornamental Plants with Ethylene Insensitivity. FRANZINE D. SMITH, R. Harriman, J. Bolar, J. Carr, D. Cobb, G. Humiston, S. Jagabeeswaran, J. Lowe, P. Popham, and N. Schneiter. Biotechnology Department, The Scotts Company, 14111 Scottslawn Road, Marysville, OH 43041. E-mail: franzine.smith@scottsco.com

In recent years, scientists have been trying to use biotechnology with traditional breeding to produce ornamental plants with more open flowers and hence more color. Of the several technologies we are using to meet this objective, here we report the use of the *etr1–1* gene of *Arabidopsis* that encodes an ethylene receptor unable to bind ethylene. Two vectors used for *Agrobacterium* transformation contained the *etr1–1* gene under the control of two different flower specific promoters. To test this approach we have produced 389 transgenic petunia lines representing four different commercial petunia varieties. Transgenic lines were confirmed by both serological and molecular assays. To date, we have screened 229 lines for extended flower life. Overall, 59% of the lines screened have flower life at least two times (2X) longer than the control and 22% have flower life three times (3X) longer than the control. Some of the lines have flower life up to 15 days compared to the controls that have an average flower life of 3 days. We have demonstrated in petunia the feasibility of tissue specific manipulation of the ethylene perception pathway to sustain more blooms, thus more color in the plants. Our next step is to screen this technology in other flowering ornamental crops.

P-1300

Analysis of Two CONSTANS-interacting Proteins of *Arabidopsis* Identified by Yeast Two-hybrid Screen. Y. H. SONG, S. H. KIM, J. S. Choi, M. J. Yi, J. Y. Park, S. Y. Shin, and J. C. Hong. Department of Biochemistry, Division of Applied Life Science, Plant Molecular Biology and Biotechnology Research Center, Gyeongsang National University, Chinju, Gyeongnam 660–701, Korea. E-mail: jchong@nongae.gsnu.ac.kr

The transition from vegetative to reproductive growth is a critical transition in the life cycle of flowering plants. The timing of flowering is one of the most important process for the reproductive success of plants. Although CONSTANS (CO) was identified as a gene controlling the photoperiodic regulation of flowering in Arabidopsis, the molecular mechanism involved in CO-mediated photoperiodic control was largely unknown. Here we report the isolation of two CO-interacting proteins (CIP1 and CIP2) from Arabidopsis using yeast two-hybrid screen. The two CIPs contains a zinc-finger motif conserved in CO and CO-like protein family by encoding 117 and 223 amino acids containing GATA-type two C_2C_2 zinc finger domain. Transgenic plants carrying sense and antisense CIP1 and CIP2 ORF under CaMV35S promoter were generated and were analyzed for phenotypic difference. The antisense suppression of both CIPs in the transgenic lines showed slightly early flowering mutant phenotype. In addition, the leaf expansion was observed even after onset of reproductive development. This indicates that these two CIPs may play a negative role in promoting flowering. (This work was supported by BK21 program and the grant from CFGC of 21C Frontier Research Program)

P-1301

Flower Color Modification of Torenia by Engineering Gene Expression of Cytochromes P450 Involved in Flavonoid Biosynthesis. K. SUZUKI, M. Mizutani, Y. Fukui, Y. Ueyama*, Y. Katsumoto, K. Miyazaki, H. Ohkawa*, T. Kusumi, and Y. Tanaka. Institute for Fundamental Research, Suntory Ltd., Osaka 618–8503, Japan and *Graduate School of Science and Technology, Kobe University, Kobe 657–8501, Japan. E-mail: Kenichi_Suzuki@suntory.co.jp

Torenia (*Torenia fournieri*), belonging to the Scrophulariaceae, is one of the most important bedding plants. Suntory Ltd. has developed a new type of torenia cultivar Summerwave (*T. hybrida*), which has many characteristics superior to common torenia cultivars. Summerwave originally has blue flowers (SWB). Because its male and female sterility predicted the difficulty of conventional breeding a molecular approach was applied to widen flower color variation. Over 80% of the total anthocyanins in the petals of SWB was malvidin 3-*O*-β-D-glucoside-5-*O*-(6-*O*-*p*-coumaroyl)-β-D-glucoside. The petals also contained malvidin 3,5-diglucoside and peonidin derivatives as minor anthocyanins. Cytochrome P450 (P450) enzymes play important roles in biosynthesis of flavonoids that determine flower color. Three P450 genes in flavonoid biosynthesis; flavonoid 3'-hydroxylase (F3'H), flavonoid 3',5'-hydroxylase (F3',5'H) and flavone synthase II (FNSII) were cloned from the torenia to modify its flower color. The transgenic torenia cosuppressed with F3'5'H gene changed flower color from blue to pink, and over-expression of F3'H gene in this transgenic plant elevated amount of cyanidin type anthocyanins and thus redder flower color. Cosuppression of FNSII gene successfully decreased amount of flavones and dramatically increased the amount of flavanones, and yielded paler flower color.

P-1302

Functional Analysis of a T-DNA Tagged Gene of *Arabidopsis thaliana*. MARIA SVENSSON[1], Dan Lundh[2], Per Bergman[3] and Abul Mandal[1]. [1]Department of Natural Sciences, University of Skövde, P. O. Box 408, SE-541 28 Skövde, Sweden; [2]Department of Computer Sciences, University of Skövde, P. O. Box 408, SE-541 28 Skövde, Sweden; and [3]Department of Plant Biology, Swedish University of Agricultural Sciences, P. O. Box 7080, SE-750 07 Uppsala, Sweden. E-mail: maria.svensson@inv.his.se

We have employed a gene knockout approach using T-DNA tagging and *in vivo* gene fusion in *Arabidopsis thaliana* for identification and isolation of specific plant genes. Screening of the T-DNA tagged lines resulted in identification of one candidate line, no. 197, exhibiting a mutant phenotype showing a three-week delay in flowering time and a tissue-specific expression of the promoterless *gus* reporter gene. A 600 bp plant DNA fragment downstream of the left T-DNA junction was cloned by inverse PCR. BLAST searching for homology identification in the *A. thaliana* genomic database indicated a putative gene, *ms197*, downstream of the T-DNA insert. We have cloned *ms197* from wild-type *A. thaliana* genomic DNA by PCR and have employed bioinformatic tools for prediction of possible protein structure and function. A fold recognition method was used to predict the protein structure. The template identified by this method indicates that the structure binds to valeric acid. Valeric acid is similar to mevalonic acid, which participates in the production of gibberellin. Thus, the results we obtained *in silico* suggest that the gene we identified by T-DNA tagging might be involved in gibberellin biosynthesis. To investigate this hypothesis we sprayed the plants of line no. 197 with gibberellic acid (GA). This treatment enhanced flowering time in the mutant plants; they flowered nearly as early as wild type plants.

P-1303

Factors Affecting Flowering in the Biennial Crucifer *Barbarea verna*. BRIAN W. TAGUE, Kendrah O. Kidd, Brian J. Ferguson, Rebecca W. Todd, Maryn E. Whittles, and Erin Davis. Department of Biology, Wake Forest University, Winston-Salem NC 27109. E-mail: taguebw@wfu.edu

An exciting avenue to explore in the post-arabidopsis genome world is the application of tools and information developed in arabidopsis to other plants with unique attributes. To this end, our lab is analyzing the signal transduction pathway regulating flowering in biennials. As a model, we have chosen the obligate biennial crucifer *Barbarea verna*. Like other obligate biennials, *B. verna* requires an extended cold treatment to flower. We have shown that *B. verna* is unresponsive to vernalization treatment until it has grown vegetatively for five or more weeks. The vernalization treatment itself must be at least five weeks long to be effective. In addition to the cold treatment, we have determined that *B. verna* requires long-days for flowering, producing an abortive inflorescence under short days. The long-day requirement for flowering can be replaced by gibberellic acid treatment. Drawing an analogy to work done in arabidopsis and other species, we are asking whether the cold treatment leads to changes in DNA methylation. Treatments with the demethylating agent 5-azacytidine did not lead to early flowering. Additional demethylating agents are currently being tested, and the degree of DNA methylation in cold- and chemical-treated plants is being measured directly. Additionally, we are generating an Agrobacterium-based transformation protocol for *B. verna*, using both floral dip and root regeneration protocols. Our goal is transform *B. verna* with constructs known to induce early flowering in *Arabidopsis thaliana* to analyze the effects of overexpression of these genes in a biennial plant. We hope to use these experiments to "map" the cold requirement in the signal transduction pathway for flowering in the biennial *B. verna*. Results of these experiments will be reported.

P-1305

Identification and Functional Analysis of a MADS Box Gene from Lily (*Lilium longiflorum*). V. A. BENEDITO[1], G. C. Angenent[1], P. B. Visser[1], F. A. Krens[1], J. M. van Tuyl[1], and S. C. de Vries[2]. [1]Plant Research International, P.O. Box 16, 6700 AA Wageningen, The Netherlands and [2]Laboratory of Molecular Biology, Wageningen University, The Netherlands. E-mail: V.A.Benedito@plant.wag-ur.nl

Lily (*Lilium longiflorum*) is one of the most important ornamental crops worldwide. Insight on the genetics of flower development in lily can drive breeding programs to produce new varieties. A cDNA library of flower buds (1–3 cm) from *Lilium longiflorum* cv. Snow Queen was constructed and 5 clones (AGLL1–5) containing sequences homologous to the *Arabidopsis* AGAMOUS (AG) gene were isolated. AG is a well-known MADS-box transcription factor that is expressed in stamens and carpels and constitutes the C-type gene in the ABC model for flower development. Clone AGLL1 has been further characterised. The clone contains a full-length sequence of 1,174 bp that is predicted to encode a 244 aa protein having 66% similarity (54% identity) to AG. The identity between both proteins in the MADS-box region is 98%. AGLL1 shares 75% similarity (64% identity) with a putative AG homologue from the monocot species *Hyacinthus orientalis*. Northern blot and RT-PCR analysis showed that AGLL1 mRNA was specifically expressed in the stamens and carpels of the lily flower bud. Overexpression of AGLL1 in Col. wild-type *Arabidopsis* showed an apetala2-like phenotype, suggesting that AGLL1 is indeed a functional AG homologue in lily.

P-1306

Attempt of an Induction of Early Flowering in Transgenic Oilseed Rape by Overexpression of the SPL3 Gene from *Arabidopsis*. M. M. WALLBRAUN, A. Biedert, M. Zwiebel, S. Härle, H. Busch*, and G. Krczal. Centrum Gruene Gentechnik, SLFA Neustadt, D-67435 Neustadt/Wstr. and *Deutsche Saatveredelung, Lippstadt-Bremen GmbH, Weissenburger Straße 5, D-59557 Lippstadt, Germany.

Modification of flowering processes is important in crop improvement. Induction of early flowering is crucial for developing crops for areas with short growing seasons. Moreover, pollination control, seed maturation and plant shape are just a few examples of traits that are dependent on flowering processes. The transition of flowering is dependent on endogenous and environmental stimuli, for example temperature, day length, light quality and availability of water and nutrients. Advances in molecular and genetic understanding of flower development provides the possibility to modify the flowering process in transgenic plants. To establish early flowering in *Brassica napus* a gene involved in floral transition that was cloned from Arabidopsis, was expressed in transgenic oilseed under control of the 35S promotor. The SPL3 gene encodes a putative transcription factor that regulates presumably a flower meristem identity gene in *Arabidopsis*. For transformation hypocotyl segments of both winter and spring rapeseed lines were co-cultivated with *Agrobacterium* strain GV3101 and were selected on DKW medium containing 5–10 mg/l phosphinothricin. PCR assays confirmed the presence of the transgene in the regenerated plantlets. The copy numbers were determined by Southern Blot analysis. It was remarkably that 20% of the primary transformants show flowering in vitro. In the greenhouse results of the flowering time of the primary transformants were promising with regard to early flowering. To verify the early flowering trait the progenies of two selected transgenic lines were investigated. Both T2 populations showed no significant changes in their flowering time in comparison to non-transformed plants. However, the activity of the transgenic SPL3 gene was shown by Northern blot analysis. The cDNA clone of the SPL3 orthologue in *Brassica napus* was isolated in a flower specific cDNA library. The different phenotypes of SPL3 overexpression in *Arabidopsis* and oilseed rape are discussed.

P-1307

Isolation of Sterile Mutants of *Verbena hybrida* Using Heavy-Ion Beams Irradiation. S. YOSHIDA[1], K. Suzuki[2], Y. Yomo[3], T. Abe[1], Y. Katsumoto[2], K. Miyazaki[3], and T. Kusumi[3]. [1]Plant Functions Lab., RIKEN, Wako, Saitama 351–0198, JAPAN and [2]Suntory Ltd., Inst. for Fundamental Research; and [3]Suntory Ltd., Flower Div. E-mail: yshigeo@riken.go.jp

Long blooming period with large number of flowers is the important characteristics of floricultural crops. A new type of Verbena cultivar 'Temari' series (*Verbena hybrida*) cultivars keep blooming with large number of flower clusters from spring until autumn in temperate zone area. However, 'Coral Pink' of this series shows sometimes the decrease in the number of flower clusters compared to other varieties. We concluded to isolate the sterile mutants of 'Coral Pink' using heavy-ion beams irradiation. Sixty four single nodes containing two lateral meristems at each base of two opposite leaves were cultured in one plastic dish, and irradiated with 1–10 Gy of the N-ion beam at 135 MeV/u. All shoots developed from lateral meristems were planted in soil, and grown in a green house. About 80 % shoots formation among all doses irradiations were observed, and most of them showed normal morphology. Some branches of flower clusters containing all sterile flowers were selectively propagated in several times by cutting. These plants were grown for flowering and the sterile strains were selected again. Finally, four mutant lines with stable sterility were successfully selected. These sterile mutants continuously grew well compared to host plant, and especially in the end of blooming season, autumn, when the host plant start senescence probably due to continuous reproductive state with seed setting. It was observed in the pot-planting test for three months that the sterile mutant always had larger number of flower clusters, finally over three times, than the host plant. Finally, we conclude that heavy-ion beams irradiation is an excellent tool for sterile mutational breeding with high frequency.

P-1308

Downregulation of Lipoxygenase Activity to Improve the Colour of Durum Wheat Flour. P. BELL, P. A. Lazzeri and P. R. Shewry. DuPont Lab, c/o Rothamsted Experimental Station, Harpenden, Herts AL5 2JQ, UK and IACR-Long Ashton Research Station, Long Ashton, Bristol BS41 9AF, UK. Email: pauline.bell@gbr.dupont.com or pauline.bell@bbsrc.ac.uk

For consumers of pasta products, perhaps the most important criterion of quality is colour; a bright yellow product being desirable. This yellowness is due mainly to the presence of a carotenoid pigment called lutein in the durum wheat grain from which pasta is made. However, some of the colour is lost during pasta processing, mainly as a result of lutein bleaching by lipoxygenase (LOX) enzymes. The impact lipoxygenases have on pasta quality make these enzymes of commercial importance to manufacturers. The reduction of their activity is therefore a suitable goal for the improvement of durum wheat. Recent research into the genetic transformation of durum wheat raises the possiblilty of specifically downregulating the expression of lipoxygenase genes, thereby reducing their bleaching activity. To this end, antisense and co-suppression vectors have been produced containing sequences from different wheat and barley LOX genes. Microprojectile bombardment has been used to transform three cultivars of durum wheat with these constructs, and several transgenic lines have been recovered. Analysis of these lines is in progress.

P-1309

Development of Transgenic Tropical Rice for Nutrition Improvement. S. K. DATTA, N. Baisakh, M. Vasconcelos, L. Torrizo, E. Abrigo, N. Oliva, I. Meinberger, M. Rai, S. Rehana, M. Arboleda, Annie B. Senner, and K. Datta. International Rice Research Institute, DAPO Box 7777, Metro Manila, Philippines in collaboration with Dr. F. Takaia, Dr. F. Goto, (Japan), P. Beyer (Germany), I. Potrykus (Switzerland). E-mail: Sdatta@CGIAR.ORG

Rice provides 40–60% of the total food calories consumed in South-East Asia. Malnutrition, particularly iron and vitaminA deficiency, is a severe problem facing many of the poor in this region. Following the work done with "Golden Rice" and high beta-carotene canola, we have developed a large number of transgenic tropical indica rice lines of different cultivars suitable to the Philippines, Bangladesh, India, Vietnam, Indonesia, etc. The transgenic plants contain three genes (*psy, crtI*, and *lcy*) for β-carotene biosynthesis in rice seeds. Further, the constructs have been modified with suitable endosperm-specific promoter(s) for enhancement of β-carotene expression in the rice endosperm. Initial screening of the primary transgenic plants through HPLC shows the expression of β-carotene in the rice endosperm. Thus far the transgenic plants have been produced with *hph* (using co-transformation) as a selectable marker gene with an aim to select the lines without *hph* (selfed out) in the subsequent generation(s). However, POSITECH selection (using *pmi* gene) is also underway as an alternative strategy of selection in rice transformation. In addition to β-carotene rice, we have also developed transgenic indica rice with *ferritin* (iron storage protein gene) and *FRO2* (gene for ferric-chelate reductase.) The genes are incorporated singly and/or in combination for stable iron enhancement in rice seeds even after polishing/milling, and for better uptake of iron particularly in iron-deficient soil. Preliminary results show an increased micronutrient content, especially iron and zinc, in both polished and unpolished seeds of the transgenics. Transgenic rice with multiple genes for nutrition biofortification including lysine (*dapA* gene in collaboration with DuPont) is also possible and will be presented.

P-1310

Transgenic 'High Protein' Sweetpotatoes (*Ipomoea batatas* L., PI-318846–3) Engineered with an Artificial Storage Protein Gene (*asp-1*) Alter the Temporal Distribution/Accumulation of Sporamin and ß-Amylase. M. EGNIN[1], M. Walker[1], C. S. Prakash[1], and J. Jaynes[2]. [1]Center for Plant Biotech Research, CAENS, Tuskegee University, Tuskegee, AL 36088 and [2]NovaTero Foundation. Email: megnin@tusk.edu

Variability of expression of native genes was analyzed in a number of ASP1-transgenic "high protein" sweetpotato lines. We have modified the nutritional quality of sweetpotato genotype PI-3188463 by using a CaMV35S driven (292bp) synthetic gene (*asp-1*) coding for a storage protein extremely rich in essential amino acids. This change in nutritional value is associated with a two to threefold increase in total protein content and essential amino acid levels in transgenics especially line TA3. All transgenic plants expressed the ASP1 protein detectable by immunoblot analysis; however, the increased protein content was primarily due to enhanced levels of native proteins such as sporamin and ß-amylase the most abundant storage proteins in sweetpotato. To investigate the molecular basis (effect of *asp-1* on) of the high accumulation of sporamin and ß-amylase in the ASP1-plants, storage roots, at four developmental stages, were taken from the "high protein" sweetpotato lines, a GUS transgenic plant, and the parental control grown under hydroponic and soil conditions. Considerable variation in the total protein in storage roots and specifically sporamin and ß-amylase expressions was observed between individual transgenics per sequential harvest in SDS-PAGE and Western blot analyses. The yield of transgenics TA3 and TA2 plants was largely comparable to the parental control. The sporamin level was found to be higher at the very early stages of TA3 and TA5 compared to TA1, TA2 and TA4 but was comparable to controls. The relative levels of sporamin gradually (temporally) increased in the developing roots of all plants. However, the sporamin content was found to be 150% to 300% (TA3) higher at maturation in all transgenic plants compared with the parental control and the GUS plant in the third and fourth harvest of hydroponic and soil cultures. Lines TA1 and TA5 had the lowest protein expression when compared to the controls at 90 days post planting (DAP). However at 136 DAP these lines accumulated up to 12 and 16% protein on a dry weigh basis, respectively. Although protein content differed significantly in all transgenics and was higher than the controls, Northern data showed that roughly equal steady state level of ASP1-transcripts accumulated in all transgenics, inferring that chromosomal position effects of ASP1-transgene and physiological determination are suggested as triggers for the variations in total protein. Sporamin and ß-amylase RNA levels were higher in the asp-1 transgenics compared to the controls and GUS transgenics. Sporamin transcripts were generally higher in TA3 than in TA2. The increase in gene expression appears to be a consequence of enhanced mRNA transcription stability rather than gene amplification because sporamin genes occur in 10 copies, per haploid genome, in ASP1-transgenic as well as non-ASP1-transgenic (GUS) and control backgrounds. This indicates that *asp-1* expression is central to the accumulation of these major native proteins in "high protein" sweetpotato revealing a temporal expression profile in all five ASP1-transgenic sweetpotato lines. *Research supported by NASA and USDA.*

P-1311

Transgenic Approaches to Making Sorghum a More Attractive Crop. I. D. GODWIN[1], S. J. Gray[1], L. Izquierdo[1], H. C. K. Laidlaw[1], S. Williams[1], J. J. Donohoe[1], P.G. Lemaux[2], and B. B. Buchanan.[2] [1]School of Land and Food Sciences, The University of Queensland, Brisbane, QLD 4072 Australia and [2]Dept of Plant and Microbial Biology, University of California, Berkeley CA 94720. E-mail: i.godwin@uq.edu.au

Sorghum is the fifth most important cereal worldwide, with 300 million people in Asia and Africa dependent on sorghum as their major staple crop. In addition to biotic and abiotic stress constraints, sorghum grain is less digestible than other cereals, which limits its attractiveness as a human or animal feed grain. Some of these traits can be approached via genetic engineering techniques. The genotypic specificity of plant regeneration has meant that reported transformation frequencies via somatic embryogenesis are low, and restricted to a few amenable genotypes. We have developed a more efficient regeneration system via organogenesis. We have used particle inflow gun (PIG) bombardment to produce fertile transgenic sorghum plants via both somatic embryogenesis and organogenesis. Transgenic plants and progenies have been produced using the *bar* selectable marker gene with selection on bialaphos. Plants with synthetic Bt genes for yellow stem borer (Chilo partellus) resistance have been produced. Current focus is on improving grain digestibility by altering the expression and anti-nutritional effects of kafirin storage proteins, and to improve the levels and stability of transgene expression. Experiments are in progress to produce transgenic plants with resistance to viral and fungal pathogens, and to overcome problems associated with cyanogenesis in vegetative plant parts.

P-1312

Selection and Regeneration of Chickpea Explants Bombarded with Desensitized Aspartate Kinase Gene. Jayanti Sen*, V. S. Reddy**, SIPRA GUHA-MUKHERJEE***. *National Centre for Plant Genome Research, **International Centre for Genetic Engineering and Biotechnology, ***School of Life Sciences, Jawaharlal Nehru University, New Delhi – 110 067, INDIA. E-mail: jayanti97@hotmail.com

Genetic transformation of chickpea using desensitised aspartate kinase (AK) gene coding for the first enzyme of amino acid biosynthetic pathway has been achieved. Following gene gun mediated transformations of explants, successful integration of pBI121 with desensitized AK gene was obtained in plants and was confirmed by PCR and Southern hybridization analysis. Transformed plants containing desensitized AK gene could also be successfully obtained after selection on kanamycin and amino acids lysine and threonine media. The lysine and threonine selection strategy, which is an antibiotic free selection, is important as it does not require presence of antibiotic genes in the construct therefore there is no need for the addition of antibiotics to the culture medium. Increase in the amounts of some amino acids such as lysine, isoleucine, aspartic acid, threonine and valine were obtained in the transgenic T0 plant leaves. An increase in the AK activity in the leaves of T0 transgenic plants was also observed.

P-1313

Zein Reduction in Transgenic Corn Plants Expressing Antisense Zein cDNAs. SHIHSHIEH HUANG, Qing Zhou, Kathleen Malloy, Whitney Adams, Dale Voyles, Jan Anthony, Al Kriz, and Michael Luethy. Mystic Research/Monsanto Company, Mystic, CT 06355. E-mail: shihshieh.huang@na1.monsanto.com.

The predominant proteins in maize seedsare a family of alcohol-soluble prolamin storage proteins called zeins, which accumulate in protein bodies during maize endosperm development. Prolamins typically contain high levels of glutamine, leucine and proline, but are nearly devoid of the essential amino acids: lysine, methionine and tryptophan. Especially among a-zeins, none of the coding sequences that have been found contain lysine. Mutations, such as opaque-2, have been shown to improve the amino acid composition and have been used to develop quality protein maize. Studies have found that the changes occurred in storage proteins of opaque-2 mutations result in a reduction in a-zein levels and an apparent increase in other lysine-rich proteins. However, many negative characteristics including soft, chalky endosperm, lower yields, and increased susceptibility to diseases and mechanical damage associated with opaque-2 have limited its application. By specifically targeting the genes that encode a-zeins, the goal of this project is to generate zein reduction corn lines with minimal side effects and, therefore, improve the amino acid composition of maize grain for human and animal nutrition. In this study, transgenic corn plants harboring various sense or antisense a-zeins constructs were shown to have reduced levels of a-zein expression and accumulation as well as the cytological changes in their endosperm. Currently, the amino acid composition of kernels produced by the transgenic plants is being determined.

P-1314

A Genetic Engineering Approach to Silencing Allergens in Peanut (*Arachis hypogaea* L.). K. N. KONAN, O. M. Viquez, and H. W. Dodo. Department of Food and Animal Sciences, Food Biotechnology Laboratory, Alabama A&M University, Normal, AL, 35762. Email: nkkonan@aamu.edu

Peanut is an excellent source of quality proteins, a health promoting food, and a favorite food for American consumers. However, peanut consumption is threatened by its allergic property. Multiple allergens have been identified in peanut. They are glycosilated seed storage proteins. Ara h 2 is the most allergenic protein triggering an IgE-mediated hypersensitive reaction in over 90% of the allergic population. The genomic DNA of allergen Ara h 2 was isolated and characterized in our Laboratory. The objective of this study is to down regulate the allergen Ara h 2 in transgenic peanut plants via the post-transcriptional gene silencing (PTGS) technology. A truncated DNA fragment of 430 base pairs long was PCR amplified from genomic Ara h 2 DNA as an *XbaI/SacI* fragment, and inserted in sense orientation into a pUC base transformation vector, between a modified CaMV 35S promoter and the NOS terminator. This construct was used with pCB13, a plasmid containing the hygromycin selection marker. Transformation was performed with the biolistic device, on embryogenic tissues of peanut variety Georgia green. PCR reactions targeting the CaMV 35S promoter, confirmed the presence of the transgene in 85% of cell lines. Southern analyses of PCR positive lines revealed the stable integration of the transgene. Northern blots in transgenic callus lines detected the presence of the transgene transcript, confirming the functionality of the transformation cassette. However, no RNA transcript from the endogenous Ara h 2 gene was detected in both the control non transformed, and transformed callus lines. We concluded that the endogenous Ara h 2 gene is not expressed at the callus stage.

P-1315

Selection of Radiation Induced 5-Methyltryptophan Resistant Mutants by In Vitro Mutagenesis and Characterization of Agronomic Traits in Rice. Y. I. LEE, D. S. Kim, I. S. Lee, D. Y. Hyun, S. J. Lee, and Y. W. Seo*. Radiation Genetic Resources Dept., Korea Atomic Energy Research Institute, Taejon, 305–600, Korea and *Division of Biotechnology and Genetic Engineering, Korea Univ., Seoul, 136–701, Korea. E-mail: yilee@kaeri.re.kr

For increasing the contents of specific free amino acids in rice (*Oryza sativa* L.) cv. Donganbyeo, the mutant cell lines resistant to growth inhibition by 5-methyltryptophan (5MT) were selected from the callus irradiated with gamma ray of 50 Gy through embryo culture. The survival rates of calli on the media containing 0.25 mM and 0.5 mM 5MT were 1.8% and 0.4%, respectively. The regeneration rates of green plants and albinoes from 5MT resistant calli were 14.8% and 0.7%, respectively. Two lines of M2–1 and M2–2 obtained from the regenerated plants by selfing were tested for 5MT resistance by M2 seed germination and growth in the 1% hyponex solution with 0.5 mM 5MT. The segregation rates of resistance and sensitivity of these lines fitted with 1:1 and 3:1 ratios, respectively. Considering the agronomic traits, M2–1 line was about the same with the original variety in culm length and a little longer or higher in panicle length and number of tillers. However, M2–2 line extremely high culm length and tillering capacity. The ripened grain ratio of M2–1 line increased significantly more than that of the original variety, although number of spikelets per panicle is a little lower, while M2–2 was quite the reverse. The grain weight of M2–1 line was almost same as the original variety, but M2–2 line was lower. Four 5MT-resistant homozygous M3 lines, M3–1–40 and M3–1–116 from M2–1, and M3–2–8 and M3–2–12 from M2–2, were obtained by selfing. Protein contents of brown rice seeds in M3–1–40, M3–1–116, M3–2–8, and M3–2–12 lines were increased about 26.6%, 13.3%, 32.35%, and 32.6% in comparison with the original variety, respectively. Total contents of 9 free essential amino acids were 84%, 86%, 42%, and 46% greater levels than the original variety in the M3–1–40, M3–1–116, M3–2–8, and M3–2–12 lines, respectively. Especially, free tryptophan levels in these selected lines were 3.4, 3.5, 2.2, and 2.1 times, and free phenylalanine levels involved in the shikimate pathway in company with tryptophan were also 2.3, 4.4, 2.6, and 2.5 times as great as the original variety. However, free tyrosine levels, the other amino acid in the shikimate pathway, in the selected lines were similar to the original variety.

P-1316

Introduction and Expression of Soybean vsp-b Gene in Maize by Genetic Transformation. Magali F. Grando*, CRISTINA D. MOREIRA**, Rex L. Smith***, Brian T. Scully***, and Robert G. Shatters, Jr.**. *Inst. Ciências Biológicas e Fac. de Agr. e Med. Vet. -Universidade de Passo Fundo, Passo Fundo-RS; ** USDA, ARS, U.S. Horticultural Research Laboratory, Fort Pierce, FL; ***Agronomy Dept. University of Florida, Gainesville, FL.

Soybean vegetative storage protein b subunit (VSP-β) assembles into a dimeric high-lysine protein that accumulates in vegetative organs before the flowering stage. During seed development, this protein is degraded to supply the seed with needed nitrogen. Research was conducted in an attempt to improve the protein concentration in silage by introducing the *vsp-β* gene in maize (*Zea mays* L.) through particle bombardment. The Hi-II/type-II callus transformation system was used as the model system to study VSP-β accumulation. Type-II embryogenic calli, initiated from immature tassels of Hi-II hybrid maize genotype, were co-bombarded with pRSVP-1 plasmid carrying the *vsp-β* gene controlled by the maize ubiquitin promoter (*Ubi-1*), and pAHC25 vector carrying the *bar* herbicide resistance selectable gene and *uidA* reporter gene. From 81 bombarded plates, 101 plants were regenerated from six independent callus lines selected on medium containing 3 mg l^{-1} glufosinate. Eighty-nine percent of these plants were resistant to 1% IgniteÓ used in leaf painting assay. The *vsp-β* gene was detected in plants derived from five lines. The co-transformation frequency of these unlinked genes was 83%. VSP-β protein accumulated to 0.6% of the soluble leaf protein in primary transgenic plants (R_0), however, the level of protein in progeny (R_1) was substantially reduced (0.03% to 0.004% of soluble protein). The VSP-β protein accumulated in leaves until seed set, after which time the level of this protein dropped to an almost undetectable level. Analysis of transcript regulation in leaves during plant development is currently underway.

P-1317

Down-regulation of Cytochrome P450-valine Genes to Obtain Acyanogenic Cassava (*Manihot esculenta*). DIMUTH SIRITUNGA and Richard T. Sayre. Department of Plant Biology, The Ohio State University,Columbus, OH 43210. E-mail: siritunga.1@osu.edu

Cassava, a root crop, is one of the most important food crops in tropical Africa, Asia and South America. Cassava leaves and roots contain potentially toxic levels of the cyanogenic glucoside, linamarin, which when hydrolyzed yields cyanide. Consumption of poorly processed cassava can cause health disorders brought on by toxic levels of cyanogens, including goiter, tropical atoxic neuropathy, permanent paralysis and, in rare cases, death. The initial, and first-dedicated step in linamarin synthesis is the conversion of valine to N-hydroxyvaline that is catalyzed by two cytochrome P450s denoted CYP79D1 and CYP79D2. Our objective has been to produce a safer cassava food product having reduced linamarin content. In addition, we are interested in analyzing the potential movement of cyanogenic glucosides between plant organs. We report here the generation of transgenic cassava plants in which the expression of the genes encoding the cytochrome P450s, CYP79D1 and CYP79D2, have been inhibited. Using a leaf-specific Cab1 promoter to drive the antisense expression of the CYP79D1 and D2 genes we observed a complete loss of CYP79D1 and D2 transcripts in leaves. Analysis of the linamarin content of leaves from six independent transformants lacking CYP79D1 and D2 transcripts indicated that there was a 80% reduction in leaf linamarin content. The same transformants also exhibit a substantial reduction in root linamarin content down to 1% of wild type levels. This is the first cassava plant that has no linamarin in its roots. In addition, we are measuring the linamarin content of leaves and roots from transgenic plants in which it is expected that CYP79D1 and D2 expression has been specifically suppressed in roots.

P-1318

Molecular Characterisation of Proline Biosynthesis Pathway in Barley: Implications for Storage Protein Synthesis and Abiotic Stress Response. E. VINCZE, S. Bowra, B. Dudas, and P. B. Holm. DIAS, Department of Plant Biology, Research Centre Flakkebjerg, DK-4200 Slagelse, Denmark. E-mail: eva.vincze@agrsci.dk

Cereals provide a very significant proportion of both human and animal diets although their nutritional quality is not optimal. One problem is that non-essential amino acids such as glutamine and proline are present in excess in the major storage proteins. These amino acids, when digested by animals, release non-utilisable nitrogen. This nitrogen is excreted in the urine, creating significant environmental load in areas where animal husbandry is prevalent. To widen our understanding about the role of proline in seed development with the aim of lowering the proline content in barley seeds, we are studying pyrroline 5-carboxylate reductase (P5CR), the last enzyme of the proline biosynthetic pathway in barley. We have isolated the P5CR coding sequence from barley cDNA library by functional complementation of an E. coli proC. The gene reported here encodes a protein with a unique chloroplast signal peptide and transmembrane domain. We will present data characterizing the clone in both transgenic and non-transgenic plants in an attempt to reconcile the conflicting pieces of evidence with respect to the subcellular localization of the P5CR enzyme.

P-1319

Super Roots in *Lotus corniculatus*: A Unique Tissue Culture and Regeneration System in a Legume Species. RYO AKASHI[1], T. Kawano[1], M. Hashiguchi[1], Y. Kutsuna[1], Shyun-Shyun Hoffmann-Tsay[2], and F. Hoffmann[2]. [1]Faculty of Agriculture, Miyazaki University, Miyazaki 889-2192, Japan and [2]Department of Developmental and Cell Biology, University of California, Irvine, CA 92697-2300. E-mail: rakashi@cc.miyazaki-u.ac.jp

Super roots of *Lotus corniculatus* are a fast growing legume root culture (Akashi et al., 1998) that permits controlled switching between root cloning and direct embryogenesis under entirely growth regulator-free culture conditions. This super-growing root culture regenerates plants that show no morphological differences as compared to wild-type regenerants or seedlings. Root branching is similar in super root and wild type plants. Roots, dissected from plantlets derived from super roots or from super root-derived protoplasts (Akashi et al., 2000), express all super root qualities again when cultured *in vitro*. Since its initiation, approximately five years ago, there is no decline in super root qualities. This extremely stable culture does not show morphological similarities with (transformed) hairy root cultures. Hairy roots are the only other root cultures known to grow with comparable vigor and continuity in growth regulator-free medium. The response to exogenous hormones is clearly different in cultures of super roots and hairy roots. Hairy root cultures do not show the regeneration qualities of super roots. Super roots are also the only root cultures allowing somatic embryogenesis or/and caulogenesis without exogenous hormone application. They, thus, provide a superior experimental system for developmental studies that are sensitive to exogenous hormones, such as lateral root formation or nodulation *in vitro*. Super roots, like all other true legume root cultures, do not nodulate *in vitro*. However, super roots carrying somatic embryos and/or small shoots, when infected with *Rhizobium loti*, form root nodules in culture. Akashi et al. (1998) Theor. Appl. Genet. 96: 758–764; Akashi et al. (2000) J. Plant. Physiol. 157: 215–221.

P-1320

Rapid Attainment of Doubled Haploids from Transgenic Maize (*Zea mays* L.) Plants by Anther Culture. I. E. AULINGER, S. O. Peter, J. E. Schmid, P. Stamp. Institute of Plant Sciences, Swiss Federal Institute of Technology Zürich, Switzerland. E-mail: ingrid.aulinger@ipw.agrl.ethz.ch

Androgenesis, the formation of homozygous plants from male gametes, has an enormous potential value for hybrid breeding since it can dramatically shorten the time required for the generation of inbred lines. Protocols for anther culture were developed in the seventies and have been improved continuously since then. In parallel to the development of the DH (doubled haploid) techniques, various systems for the genetic transformation of maize were established using protoplasts, zygotic embryos, embryo derived callus or cell suspensions as target material. In comparison to inbred lines, hybrids show a superior performance in cell- and *in vitro* culture. Thus, most publications on maize transformation report about the transformation of heterozygous material. Consequently, the resulting progenies segregate not only for the transgene but also for the genetic background, causing a high degree of phenotypic variation. The development of a homozygous transgenic line from such material by conventional back crossing is time consuming and requires a selection for the transgenic trait in each generation, before its integration into a hybrid breeding programs is feasible. In our lab we attempted to obtain fully homozygous transgenic DH lines in two subsequent steps from anther cultures initiated with heterozygous transgenic donor material. We inter-crossed the transgenic genotype 109.2, with the androgenic genotype ETH-M82. 109.2 is segregating for the herbicide resistance gene pat and was obtained by particle bombardment of zygotic embryos derived from the hybrid A188 x H99. The offspring exhibiting herbicide resistance was used as anther donor plants. Almost 6000 anthers were isolated and gave rise to more than 160 DH regenerants. Transgenic DH regenerants were then self-pollinated in order to establish a transgenic homozygous line. Phenotypic and genotypic analysis of progenies revealed the complete homogeneity of the material and the effective diploidization by the anther culture method. By this procedure we were able to obtain a pure transgenic line within one year.

P-1321

Importance of Isolated Microspores Culture in the Regeneration of Embryos in *Coffea arabica* cv Catimor. MARIA F. CARNEIRO[1] & Isabel R. Moura[2]. [1]Centro de Investigação das Ferrugens do Cafeeiro/IICT, Quinta do Marquês 2784–505, Oeiras Portugal; [2]Jardim-Museu Agrícola Tropical/IICT, Largo dos Jerónimos, 1400–209 Lisboa, Portugal. E-mail: mf.carneiro@clix.pt

A large work has been done in order to obtain haploid plants from *Coffea arabica* cv Catimor resistant to coffee main diseases, culturing isolated microspores or anthers. The main advantage of haploids is that they can be used to produce rapidly homozygous lines. In culture, microspores undergo different pathways of androgenesis which lead to the formation of haploids either directly by embryogenesis, or indirectly via callus formation. The experiments were carried out with some progenies of cv Catimor. The anther developmental stage and gametophytic answer of microspores during the culture was checked, using two methods, acetocarmine and DAPI (4,6-Diamino-2-phenyldole.2HCL). The viability of microspores was determined using Diacetate of Fluoresceine (FDA) test. In previous experiments anther/microspores culture was performed. Although, the ability of whole anthers to induce androgenesis is the same of the isolated microspores/pollen grains, there is a meaningful difference in the time and efficiency of the isolated microspores /pollen grains to achieve the phase of embryos. In this communication we will present some results obtained using two techniques of microspores extraction: Nitsch and Shed Pollen. The isolated microspores when Nitsch technique was used were cultivated in MS and Nitsch liquid media supplemented with different growth regulators and vitamins and the cultures were cultivated in stationary or shaking liquid media. When Shed pollen technique was used the extraction of the microspore was achieved with periodic transference of the anthers to fresh liquid MS and Nitsch media. Using these techniques different phases of embryogenesis were achieved in a short period of time.

P-1322

Anatomical and Morphological Study of the Flower Development in Carob Tree (*Ceratonia siliqua* L.). L. CUSTÓDIO, M. F. Carneiro* and A. Romano. Faculdade de Engenharia de Recursos Naturais, Universidade do Algarve, Campus de Gambelas, 8000–117 Faro, Portugal. *Centro de Investigação das Ferrugens do Cafeeiro, 2784–505 Oeiras, Portugal. E-mail: aromano@ualg.pt

Carob tree is polygamo-trioecious, with male, female and hermaphrodite flowers, exhibiting high plasticity in inflorescence and flower characteristics. The development of male and hermaphrodite flowers comprehends five stages, each one exhibiting proper morphological and anatomical characteristics. An anatomical and morphological description of the anthers from the first stages of differentiation of the flower bud until dehiscence, is presented. The histological study showed that on phase 0 the anthers' tissues were not yet differentiated and were completely surrounded by sepals possessing trichomes. In the early stages of phase I we clearly distinguished an epidermal layer, an outermost wall layer, the endothecium and the connective. The sporogenous tissue is developed in the four angles of the developing anther. The inner layer of these initials constitutes the primary sporogenous cells, which will form the pollen mother cells. In the final stages of phase I the anther is bilocular, each locule containing two pollen sacs completely differentiated by this time, and covered in the innerside by the tapetum. The tapetum apparently serves for the nourishment of the developing pollen mother cells and microspores, already visible by this time. Transversal sections of anthers on phase II revealed a similar structure that those of final stages of phase I, but by this time the pollen sacs are open and the tapetum cells begun disintegration. In phase III dehiscence has already occurred and one can observe disintegrated fragments of tissues and many pollen grains.

P-1323

Somatic Fusion Between Eggplant (*Solanum melongena*) and Sexually Incompatible Arboreous Solanum Species. A. FURINI(1), L. Borgato(1) and F. Salamini(2). (1) University of Verona, Dept. of Science and Technology, Strada Le Grazie, 15 37134 Verona, Italy and (2) Max-Planck-Institut, Carl-von-Linne'-Weg 10 50829 Koeln, Germany. E-mail: furini@sci.univr.it

Because of the high susceptibility of eggplant to soil pathogens, up to now the aim of somatic hybridization between *S. melongena* and wild *Solanum* species was to transfer pathogen resistance traits. *Solanum* is one of the largest genera of vascular plants consisting of more than 1500 described species. Therefore, it represents a source of variability that may be explored for the genetic improvement of many different traits. The cellular fusion between the herbaceous cultivated eggplant and wild *Solanum* species with arboreous growth habit is the aim of this research. *S. linnaeanum*, *S. macrocarpon* and *S. virginianum* have been selected for they arboreous and woody growth and although they have genetic affinity with the cultivated eggplant they showed sexual incongruity. Protoplast fusion have been achieved by PEG and electrofusion and heterokaryons have been visualized by fluorescent labeling of parental protoplasts and selection of dual-stained heterofusion products. Plant regeneration from microcalli may result not only in the regeneration of plant characterized by hybrid vigor, but also by different plant architecture with arborescent or shrubby habitus. The new varieties may have perennial instead of annual fruit production.

P-1324

Establishment of a Transformation System of Pearl Millet (*Pennisetum glaucum*). M. GIRGI, A. Morgenstern*, K. H. Oldach**, and H. Lörz. UHH Institute of General Botany, Applied Molecular Biology of Plants (AMPII), Ohnhorststrasse 18, 22609 Hamburg, GERMANY. Email: fb0a009@botanik.uni-hamburg.de

Pearl millet, a diploid C4-cereal, is one of the main crops in semi-arid regions and the sixth most important one world-wide. It is tolerant to drought and to very acid soils and can be grown in low rainfall areas where maize and sorghum are not profitable. Pearl millet is a palatable, high-yielding summer forage. Its seeds contain 70% starch, 16% proteins and 5–7% fat. High values of essential amino acids, vitamin A, calcium and iron characterise this cereal. Although resistant against many diseases, pearl millet is susceptible to several fungal pathogens, i.e. smuts (*Helminthosporium* spp.) and downy mildew (*Sclerospora graminicola*). The permanent increase of the world population and the expansion of deserts endanger human nutrition. Due to its good adaptation to drought and heat, pearl millet is an important crop to help attain food security where other cereals fail. Therefore it is of great interest to develop high yielding and pathogen resistant cultivars. In addition to classical breeding, genetic engineering is a promising strategy to introduce valuable traits into pearl millet. For genetic enhancement two preconditions have to be fulfilled: (1) an efficient *in vitro* culture- and (2) an efficient transformation-system. For the establishment of the *in vitro* culture system different parameters were tested to determine the optimal culture conditions and the initial explant material. Best plant regeneration rates were achieved with immature embryos (0,5–1,2 mm). After varying different media, carbon source and hormone concentration (auxine/cytokinine-relation), modified L3-medium*** showed highest number of regenerated explants. For the transformation of pearl millet the biolistic method was applied using visible (*uid*A) and selection marker (*bar*) genes. Immature embryos were bombarded with gold particles (0.4–0.8 µm) at a pressure of 1550 psi. First transformants were obtained about six months after bombardment. Integration and expression of transferred genes was confirmed by histochemical GUS staining and Southern blot analysis.

P-1325

Influence of the Chosen Factors on the Androgenesis in Anther Cultures of Carrot. KRYSTYNA GÓRECKA, Dorota Krzyżanowska, Elżbieta U. Kozik, and Renata Nowak. Research Institute of Vegetable Crops, 96–100 Skierniewice, Poland. E-mail: kgoreck@inwarz.skierniewice.pl

The effectivity of embryogenesis in carrot anther cultures was examined under different factors: a/ term of sowing for obtaining donor plants (two terms), b/ method of managing of donor plants c/ growth conditions of them. ad. b/ The donor plants were managed in 4 different manners: 1) only one shoot and only one main inflorescens from one root were allowed to develope; 2) one shoot one main umbel and a few umbels of the first order; 3) few shoots growing up from the root and only one main umbel on each of them; 4) the plants were not pruned. The donor plants were grown in ordinary greenhouse and in the open field. Higher number of embryos per 100 anther (3,1) were received from the donor plants which were sown in the first term and 2,6 from the plants sown in the second term. The percent of reacting anthers was respectirely 2,6 and 1,7. The highest number of embryos and the percent of reacting anthers were obtained from plants with 1 stem one main umbel and some umbels of the first order respectively 3,8 and 2,7. In the anther cultures initiated from the plants which were cultivated in greenhouse 0,5 embryos per 100 cultured anthers were obtained in the fewest embryogenic variety and 46,5 in the most. The anthers taken from the donor plants of the some varieties grown in the open producted 0,0 and 33,5 embryos per 100 anthers. The donor plants from the two terms of sowing gave similar numbers of embryos and the percents of responsive anthers. The method of managing of donor plants hadn't considerable influence on the effectiveness of embryogenesis in the anther cultures. Each the tested varieties gave better yield of embryos and higher percent of reacting anthers, when they were grown in greenhouse. Marked differences were found between searched varieties in their ability of embryos production in anther cultures.

P-1326

Molecular Studies on Development of Pollen Embryos in Tobacco and *Brassica*. SIPRA GUHA-MUKHERJEE and Semarjit Shary. Plant Reasearch Laboratory, School of Life Sciences, Jawaharlal Nehru University, New Delhi 110 067, India. E-mail: sipra@hotmail.com

Factors influencing totipotency of pollen grains of tobacco and *Brassica* have been studied. Both tobacco and *Brassica* are amenable materials for induction of pollen embryos. Tobacco has been used as a model plant to study various factors which could change the developmental pathway of pollen grains. Molecular studies in these plants have shown that certain genes are up/down regulated during pollen embryogenesis in response to specific signals. Some of these genes can be used as molecular markers for identification of potential for androgenesis of a plant. During callusing of microspores a completely new set of genes are triggered. Differential display and subtraction hybridization techniques were used for identification of genes, specifically expressing during pollen embryogenesis. Sequences of these genes show homology with a few of known genes such as cyclin whereas others are considered as novel genes. These are found to be developmentally stage specific.

P-1327

Organelle DNA Transmission of Intergeneric Somatic Hybrids of *Citrus* with Its Related Genera Revealed by CAPS and RFLP Analyses. W. W. GUO, Y. J. Cheng, and X. X. Deng. National Key Laboratory of Crop Genetic Improvement, Huazhong Agricultural University, Wuhan 430070, China. E-mail: GWW@LAL.UFL.EDU

Cleaved amplified polymorphic sequence (CAPS) and restriction fragment length polymorphism (RFLP) were successfully applied to analyse the organelle DNA transmission of two intergeneric somatic hybrids between *Citrus* and its related genera, i.e. Valencia orange (*Citrus sinensis* (L.) Osbeck) + Meiwa kumquat (*Fortunella crassifolia* Swingle), red tangerine (*C. reticulata* Blanco) + trifoliate orange (*Poncirus trifoliata* (L.) Raf.). Five chloroplast and three mitochondrial universal primer pairs were used, and the PCR products were digested by fifteen restriction endonucleases respectively. Four polymorphic cpDNA-CAPS and two mtDNA-CAPS markers were found in both somatic hybrids. The results showed that both cpDNA and mtDNA in the four tested plants of Valencia + Meiwa somatic hybrids were derived from Valencia embryogenic suspension. While in the four tested plants of red tangerine + trifoliate orange somatic hybrids, their cpDNA were all from trifoliate orange (the leaf parent), and mtDNA from red tangerine (the embryogenic suspension). Genomic DNA of the Valencia + Meiwa somatic hybrids and corresponding parents was digested by five restriction endonucleases, and hybridized with one chloroplast and eight mitochondrial probes. The results were in line with those of CAPS, and non-parental novel bands were detected, which indicated that various organelle DNA recombination or rearrangements occurred in the hybrids. Moreover, great differences among the four individuals under the same nuclear background were found. The banding pattern of organelle genomes at the molecular level was correlated with their growth vigor, which suggested that the nuclear-cytoplasm incompatibility or the organelle DNA instability existed. Supported by National Natural Science Foundation of China (NSFC), the International Foundation for Science (IFS), and by Chengguang Youth project of Wuhan City in China.

P-1328

In Vitro Androgenesis in Apple. MONIKA HÖFER[1], Alisher Touraev[2], and Erwin Heberle-Bors[2]. [1]Federal Centre for Breeding Research on Cultivated Plants, Institute for Fruit Breeding, Pillnitzer Platz 3a, D-01326 Dresden, Germany, and [2]Vienna Biocenter, Institute of Microbiology and Genetics, University of Vienna, Austria. E-mail: M.HOEFER@BAFZ.DE

Microspore systems offer new possibilities for genetic studies and the application of sporophytic and gametophytic pathways in breeding research. Recently, a protocol of microspore culture was developed in apple (*Malus domestica* Borkh.) based on an optimized isolation technique, and successful plant regeneration was obtained from isolated microspores (Höfer et al., 1999). The embryo induction phase was optimized regarding the pretreatment of microspores for initiation of microspore embryogenesis, the induction medium and the concentration of microspores in the suspension. Furthermore, the effect of the donor genotype was studied. To determine the efficiency of *in vitro* androgenesis both ways, the anther and microspore culture, were investigated by using the same experimental bud material. Regarding the dependence of the genotype and the variation of the donor material, the same tendencies could be observed. Comparing the efficiencies of embryo induction in anther and microspore cultures, microspore culture resulted in an increase up to 10 times depending on the genotype. The regeneration process in microspore culture is similar to that of androgenic embryos via anther culture and demonstrates an adventitious shoot formation in most cases after a long period of secondary embryogenesis. A final calculation of the efficiency of both methods is in progress. At present, the first lines derived from anther and microspore culture exist after grafting in the orchard.

P-1329

New Strategies for Creation of Basic Material of Various *Brassica* species with Resistance to TuMV. E. KLOCKE[1], Pham T. L. Thu[2], U. Ryschka[1], J. Schubert[1], R. Krämer[1], G. Schumann[1]. [1]Federal Centre for Breeding Research on Cultivated Plants, D-06484 Quedlinburg, Germany and [2]Institute of Agricultural Genetics Tuliem, Hanoi, Vietnam. E-mail: E.Klocke@BAFZ.de

Turnip mosaic potyvirus (TuMV) causes losses in yield and quality of *Brassica* sp. Since sources of resistance to this virus are limited use of pathogen derived resistance (PDR) offers a promisingly alternative. It could be realized by using gene transfer. For introduction of resistance genes of coat protein and NIB of TuMV have been used. Transgenic plants of *Brassica oleracea* var. *botrytis* 'Korso' and *B. napus* 'Hanna' and 'Maplus' were obtained through PEG-mediated direct transformation of hypocotyl protoplasts. The protoplasts were transformed by using one or two plasmids. In experiments with two plasmids one plasmid (pGl 2) contained the selective gene *hpt*. Plants were regenerated with selection agens hygromycin. Furthermore, without selection it could be obtained besides non-transgenic plants transgenic plants too. PCR and Southern hybridization confirmed the presence of the foreign genes in the plant genome. First regenerates checked by DAS-ELISA showed various levels of resistance to different pathotypes of TuMV. In generally, the results demonstrate that the direct gene transfer via protoplasts proposes new possibilities for transfer of few genes coincidentally and independently.With regard to biosafety the described results could be useful. There is the chance that the transgenic plants contained only the gene of interest either caused by independent transfer of genes or by using a protocol without selection pressure.

P-1330

Production of Interspecific Somatic Hybrids Between Transgenic *Lycopersicon esculentum* Beta 11 Variety and *Solanum lycopersicoides*. M. KULAWIEC(1), R. Sniezko(1), S. Malepszy(2). (1)Department of Cell Biology, Maria Curie Sklodowska University, Akademicka 19, Lublin, Poland and (2) Department of Genetics, Breeding and Biotechnology, Warsaw Agricultural University, Nowoursynowska 166, Warsaw, Poland. E-mail: markul@excite.com

Our hybridization experiment was aimed to produce somatic hybrids which can be used to obtain a new tomato form well adopted to environmental and agricultural conditions in Poland. The Beta 11 variety tomato recommended to direct consumption and wild *Solanum lycopersicoides* were used. Transgenic line of Beta 11 var. tomato with introduced thaumatin gene and *npt II* gene for neomycin phosphotransferase was obtained via *Agrobacterium tumefaciens* transformation in the Deptartment of Genetics Warsaw Agricultural University. *Solanum lycopersicoides* is a wild, diploid species that carries chilling temperature tolerance and resistance for pathogens. Sexual hybridization of these species is not efficient. That is why somatic hybridization has been used to transfer the resistance to low temperature and pathogens into tomato germplasm. Leaf mesophyll protoplasts of tomato were fused using modified PEG/DMSO procedure with suspension culture-derived protoplasts of *Solanum lycopersicoides*. Identification of fusion product was due to double yellow-red fluorescence Heterocaryons were obtained with the frequency of 0,1–6,0%. Protoplasts were plated in modified 8E medium and 2,5D+10E medium. After kanamycin was added to the tested media, *Solanum lycopersicoides* protoplasts were eliminated. Tomato protoplasts were not able to grow in the tested conditions, so only putative hybrid microcalli, due to the presence of *npt II* gene from transgenic tomato and regeneration abbility of *Solanum lycopersicoides*, developed in the post fusion culture. Tetraploidy of these calli was revealed by flow cytometric analysis. PCR and Southern hybridization analysis confirmed the presence of taumatin marker gene originating from the tomato transgenic line. Shoot initiation and regeneration of the whole plants was carried on MS3ZG medium. Putative hybrid regenerants were transferred to the greenhouse after 3 months. Morphological investigations of the somatic hybrids plants reveal morphological features of both parents. GISH analysis of tomato (+) *Solanum lycopersicoides* will be performed to confirm hybridity.

P-1331

Transient Expression of PEG-mediated Gene in Mesophyll Protoplasts of Pepper (*Capsicum annuum* L.). J. M. Jeon, Y. I. Ha, H. K. Joung, Y. S. Lee, Y. J. Choi*, C. O. Lim, and S.-H. LEE. Division of Applied Life Science, Gyeongsang National University, Jinju 660–701, Korea. *Department of Food and Nutrition, Silla University, Pusan 617–736, Korea. E-mail: leesh@nongae.gsnu.ac.kr

The genus *Capsicum* of the nighshade family Solanaceae includes five major cultivated species. Among them, *Capsicum annuum* L. (pepper) is the most extensively cultivated species and an economically important vegetable and spice crop in Korea. Although in vitro plant regeneration from cotyledon and hypocotyl of pepper via organogenesis has been reported, no fertile transgenic peppers have yet been developed using protoplast system with PEG-mediated gene transfer. Therefore, we are interested in PEG-mediated genetic transformation of pepper with the aim of producing disease resistant transgenic plants. Protoplasts were freshly isolated from leaf tissues of *Capsicum annuum* L. Yields of protoplasts ranged between 1.5×10^6 and 7.5×10^6 protoplasts/g FW depending on enzyme concentration and incubation time. An overnight exposure for 12 h to cell wall digesting medium containing 1.2% cellulase R10 and 0.3% macerozyme R10 resulted in enhanced yield and ensured complete digestion of cell walls. The level of transient expression of the gus gene demonstrated the efficiency of applied concentration and molecular weight of PEG and incubation time to introduce foreign genes into leaf protoplast of pepper. These must exceed a certain threshold value in order to permeabilize the plasma membrane and facilitate DNA uptake. As far as the level of transient gene expression is concerned, higher concentration of PEG and lower molecular weight of PEG favoured higher gene expression—the optimum being at 40% concentration and 3,350 molecular weight of PEG solution having 30 min of incubation time. The 6 lines of transformed protoplasts were analyzed biochemically. Genomic DNA extracted from 6 lines of transformed protoplasts and vector control were analysed for the presence of the *gus* gene by PCR using *gus* specific primers. A 1.5 kb fragment of the *gus* gene was amplified by PCR from undigested pBI121(positive control) and 6 lines of transformed protoplasts. DNA extracted from nom-transformed control protoplasts and water control did not show any amplification of the *gus* gene by PCR. To introduce foreign gene into pepper protoplasts, we established the optimum transformation conditions using transient expression of gus gene. Therefore This method applied to introduce into pepper protoplasts with other plasmid DNA containing GFP gene. The result also appeared, as the shown gus gene expression.

P-1332

An Efficient System for Protoplast Culture from Alfalfa (*Medicago sativa*) Suitable for Plant Transformation and Regeneration. V. LEVEE, M. Bertrand, M. Duval, and L.-P. Vezina. Medicago Inc., 1020 Route de l'Église, Bureau 600, Sainte Foy, Québec, Canada, G1V 3V9. Email: leveev@medicago.com

Medicago inc is committed to the development of cost-efficient methods for the use of alfalfa as a cellular factory for pharmaceuticals. The species has been chosen for its high protein content, high yield, numerous environmental benefits and perennial characteristics Although Agrobacterium-mediated transformation has proven efficient on regenerative genotypes, the method has limitations in the context of recombinant product development. Although use of more direct DNA transfer methods is appealing, it is limited by the inefficiency of plant regeneration from isolated cells or protoplasts. We have developed an improved method to purify and regenerate protoplasts from leaf tissue of alfalfa. With this improved system which comprises new medium composition for digestion, purification and initial development, first cell division occurs at day 7 and ten-cell microcolonies have formed within 2 weeks of protoplast isolation. Within 3 to 4 weeks, 20% of protoplasts have developed colonies of 50–100 cells. These colonies are transferred to regeneration conditions in which 5% of protoplasts develop into microcalli within 6 weeks. Most microcalli then form somatic embryos from which plantlets are obtained 15 weeks after protoplast isolation. Performance of this method was established on protoplast which were isolated at the earliest stage in agarose films. Results on colony and microcallus development from protoplasts will be discussed as well as applications of this method for high throughput genetic transformation.

P-1333

Production and Characterization of Interspefic Asymmetric Somatic Hybrids via Donor Recipient Fusion in *Citrus*. JIHONG LIU and Xiuxin Deng. National Key Laboratory of Crop Genetic Improvement, Huazhong Agricultural University, Wuhan 430070 P. R. China. E-Mail: JHLIU26@public.wh.hb.cn

Embryogenic protoplasts of Valencia sweet orange (*Citrus sinensis* Osb.), irradiated with X-ray for 45 min at 5 mA and 80 kVp, were electrofused with embryogenic protoplasts of Murcott tangor (*C. reticulata* × *C. sinensis*) that were treated with 0.25 mM iodoacetic acid (IA) for 15 min. IA treatment could arrest mitotic division of the protoplasts of Page completely and the protoplasts broke under current culture system. Division of Valencia protoplasts irradiated for 45 min was not prevented but could be delayed by one week or so. However, no callus could be obtained from the irradiated protoplasts. The fusion-treated protoplasts could develop into embryoids only when the callus derived from fusion treated cultures was transferred to a medium containing MT supplemented with 2% glycerol, from which normal shoots could be developed. But the shoots were quite recalcitrant to rooting in the root induction medium. Only one shoot could root. Therefore *in vitro* grafting was employed to obtain complete plants. Cytological observation showed that the plants contained mainly diploid and aneuploid cells (72.9% and 22.9%, respetively), together with very few tetraploid cells (4.2%), indicating that extensive chromosome elimination has happened. The results implied that the plants were neither diploid nor tetraploid, but mixoploid. Random amplified polymorphic DNA analyses with three 10-mer arbitrary primers confirmed the plants as true somatic hybrids. Combination of cytological and molecular analysis showed that the plants were asymmetric somatic hybrids derived from donor recipient fusion between the interspecific combination.

P-1334

Green Fluorescent Protein as Visual Marker in *Citrus* Somatic Hybridization. O. OLIVARES-FUSTER and L. Navarro. Department of Plant Protection and Biotechnology, Instituto Valenciano de Investigaciones Agrarias, Moncada, Valencia 46113, Spain. E-mail: oscar@ivia.es

Suitability of the Green Fluorescent Protein as *in vivo* marker to follow the overall process of protoplast fusion, regeneration and selection of hybrid plants has been studied using a transgenic citrus plant expressing the Green Fluorescent Protein as parent in somatic fusion experiments. A high level of Green Fluorescent Protein expression was detected in citrus transgenic protoplasts, hybrid callus, embryos and plants. Green Fluorescent Protein can be used for the continuous monitoring of the fusion process, localization of hybrid colonies and callus, and selection of somatic hybrid embryos and plants.

P-1335

Somatic Hybrids of Oilseed Rape *Brassica napus* L. W. ORCZYK. Plant Breeding and Acclimatization Institute, Radzikow, 05–870 Blonie, Poland. E-mail: W.ORCZYK@IHAR.EDU.PL

Protoplasts isolated from male sterile (CMS Ogura) and male fertile rapeseed (*Brassica napus* L.) were fused with PEG. Prior to fusion protoplasts from one component were irradiated with UV or pretreated with iodoacetamide or chloramphenicol. Plants regenerated after protoplast fusion were analyzed for their phenotype (type of flowers, number of nectaries, chlorophyll content), molecular (cpDNA and mtDNA, number of chromosomes, relative DNA content) and physiological traits (gas exchange parameters, intensity of photosynthesis). Organellar DNA was analyzed by means of PCR reaction with random and specific primers. Randomly amplified fragments were specifically hybridized with probes based on cpDNA. Combination of both techniques (RAPD with DNA hybridization) revealed cpDNA changes in plants obtained after protoplast fusion. Primers designed for specific regions of mitochondrial DNA revealed that certain mtDNA molecules were present in substoichiometric quantities. About 80% of plants regenerated after protoplast fusion were identical to one of the fusion components and about 20% had modified/recombined traits. Over 3/4 of changed plants were derived from experiments where protoplasts were treated with iodoacetamide, chloramphenicol or were irradiated with UV. This indicated the efficiency of selection of heterokaryons after application of one of these treatments. Regenerated plants developed male fertile or male sterile (CMS Ogura type) flowers. Part of male sterile plants had elevated, comparing with CMS Ogura, chlorophyll content under lower temperature conditions. Some of them had four nectaries and higher nectar secretion then CMS plants. Molecular analysis confirmed the presence of changes in chloroplast and mitochondrial DNA. Some plants had recombined chloroplast DNA. Gas exchange parameters (photosynthesis intensity) were not correlated with lower chlorophyll content in male sterile or male fertile plants growing under lower or normal temperature. This indicated that chlorophyll content was not the limiting factor of photosynthesis intensity of analyzed plants.

P-1336

Improved Plant Regeneration from Anthers of Dihaploid Rye (*Seacale cereale* L.). MONIKA RAKOCZY-TROJANOWSKA and Maja Lewandowska. Department of Plant Genetics, Breeding and Biotechnology, Warsaw Agricultural University, Nowoursynowska 166, 02–787 Warsaw, Poland. Email: rakoczy@alpha.sggw.waw.pl

Winter rye (*Seacale cereale* L.) is a typical heterozygotic, self-incompatible species. Thus, the production of inbred lines, valuable for different breeding purposes, is highly limited. Haploids could be an excellent source of obtaining homozygotic forms. However, in spite of many attempts, no spectacular results for winter rye have been published. Somaclonal variation, a phenomenon occurring during plant regeneration *in vitro,* concerns wide range of characters. It can be also expressed as modified *in vitro* response. Therefore, we decided to examine the regeneration ability of anthers isolated from dihaploid (DH) plants obtained previously. Eight DH/F_2 families (derived from highly homozygotic line L318 bred in our Department) were used as donor materials. The percentage of anthers producing embryogenic callus – 1.28% was comparable with the control (anthers of line L318) and with the results of our previous experiments. Finally, 21 green plants were regenerated from 9352 anthers of DHs and 1 plant from 2000 anthers of control line. The regeneration rate counted for DHs – 0.23% was significantly higher than for the control and nearly 10 fold higher than we have ever obtained in our previous experiments. The results obtained in this work indicate that anther culture derived plants may have improved potential of haploid production, which is especially important for such a species like rye. This approach might be also used to obtain plant material suitable for molecular mapping. This work was partially supported by grant No. 6 PO6A 007 20 from State Committee for scientific Research (M. R.-T.).

P-1337

In Vivo Microsporogenesis in Carob Tree (*Ceratonia siliqua* L.). L. CUSTÓDIO, M. F. Carneiro*, and A. Romano. Faculdade de Engenharia de Recursos Naturais, Universidade do Algarve, Campus de Gambelas, 8000–117 Faro, Portugal and *Centro de Investigação das Ferrugens do Cafeeiro, 2784–505 Oeiras, Portugal. E-mail: aromano@ualg.pt

The pollen developmental stage when the flower bud is cut from the donor plant is critical for successful androgenesis. In order to establish a correlation between the stages of flower and microspore development, an *in vivo* study was carried out to follow the development of pollen and anthers of carob tree from the first stages of differentiation of the flower bud until dehiscence. Using two staining methods, acetocarmine and DAPI (4-6-Diamidino-2-phenylindole.2HCl), it was possible to clearly visualize the process of microsporogenesis. This process may be considered as normal, since it exhibits all the characteristics of the gametophic development of the angiosperm. In phase 0, we can observe primary sporogenous tissue and pollen mother cells also known as microsporocytes. On the early stages of phase I there were pollen mother cells, tetrads of microspores surrounded by callose and isolated microspores. In the final stages of this phase we observed mainly isolated uni and binucleated microspores and some initiating division. In phase II microspores looked similar to those observed in final stages of phase I, but there were already completely formed pollen grains. Finally, in phase III, corresponding to the starting of anther dehiscence stage only mature pollen grains were observed. The observations carried out proved the close relationship between the development of the anther and the pollen grain formation in carob tree. The study of the *in vivo* microsporogenesis process in carob tree will allow to follow all the aspects related to the development of pollen *in vitro*, and to understand not only the androgenic process but also other important aspects of its cellular biology.

P-1338

Correlation of Ploidy Level and Phenotype in *Physcomitrella patens*. G. SCHWEEN, A. Hohe, J. Schulte, R. Reski. Plant Biotechnology, Freiburg University, Sonnenstrasse 5, D-79104 Freiburg, Germany. e-mail: schween@uni-freiburg.de homepage: www.plant-biotech.net

The haploid moss *Physcomitrella patens* is the only plant showing high rates of homologous recombination in its nuclear DNA, making it possible to identify gene function by targeted knockout. The current production of a saturated mutant collection is based on PEG-mediated protoplast transformation and after regeneration and selection on antibiotic-containing medium the ploidy level of each stable transformant is determined by flowcytometry and the phenotype is characterized and documented. The ploidy level of 24,400 transgenic Physcomitrella plants has been tested so far and 8.8 +/- 5.4% were polyploid (2n = 8.7% and 4n = 0.1%), probably due to protoplast fusion during the trans-formation procedure. To check whether variation in moss phenotype could be induced not only by gene knockout but also by changes of the ploidy level, ploidy and phenotype of 418 haploid and 80 diploid untransformed regenerated moss plants were correlated. Diploid plants showed retarded growth on Knop medium, a reduced number of gametophores and changes in the form of the gametophores. Phenotypic deviations in more than one characteristic strongly indicated polyploidization. Correlation coefficients for ploidy level and these features were between 0.5 and 0.7 for the 500 plants that had been regenerated after mock-transformation as well as for the first 10,000 stable transformands of our mutant collection. Acknowledgement: This work has been performed in a joint project with BASF Plant Science GmbH.

P-1339

The Influence of Pretreatment on the Time of DNA Synthesis in Barley (*Hordeum vulgare* L.) Uninucleate Microspores. YOUN-SEB SHIM and Ken J. Kasha. Department of Plant Agriculture, University of Guelph, Guelph, ON, N1G 2W1, Canada, E-mail: yshim@uoguelph.ca

This research was undertaken to improve our understanding of the process of development of microspores leading to embryogenesis and haploid plants. It also should help to determine the optimal time for the bombardment of microspores relative to cell cycle in order to produce homozygous transgenic plants. Based on comparative DNA densities of microspores, we attempted to correlate the time of first DNA synthesis in haploid microspores of barley with morphological stages and to subsequently assess the influence of pretreatment on DNA synthesis at different stages of microspore development. Microspores in early(E), mid(M), and late(L) uninucleate stages were isolated from the barley cultivars Manley and Igri, and subjected to two commonly used pretreatments. First, during cold pretreatment for 28 days, there was a slow increase in relative DNA values as well as greater variation in DNA values at a microspore stage. Second, during a cold plus mannitol pretreatment, there was very little change in the microspore stage or DNA values indicating that this pretreatment blocked the progression of the cell cycle at all stages both of Igri and Manley. During cold pretreatment the majority of microspores reached the L uninucleate stage. It was concluded that microspores in the L stage had undergone DNA synthesis and were at the G2 stage. These studies indicated that only the cold plus mannitol pretreatment blocked or delayed microspore development at all the cell cycle stages. This information will form the basis for subsequent studies including gene transformation by particle bombardment of regenerable tissues.

P-1340

Somatic Hybridization of *Brassica napus* L. with Brassicaceae for the Improvement of the Fatty Acid Composition. K. SONNTAG, I. Groeneveld, E. Rudloff, and J. Gramenz. Federal Centre for Breeding Research on Cultivated Plants, Institute of Agricultural Crops, Rudolf-Schick-Platz 3a, 18190 Groß Lüsewitz, Germany. E-mail: k.sonntag@bafz.de

Somatic hybridization of *B. napus* with selected Brassicaceae provides the possibility to combine high yield and ecological adaptability of *B. napus* with valuable traits of other Brassicaceae, e.g. resistance to pests or a high content of erucic acid. After development and adaptation of methods for isolation, fusion and regeneration of mesophyll protoplasts it was possible to obtain somatic hybrids of *B. napus* (+) *B. juncea* L. (brown mustard), *B. napus* (+) *Raphanus sativus* L. var. *oleiferus* (oil radish) und *B. napus* (+) *Sinapis alba* L. (white mustard). Identification of the regenerated shoots as somatic hybrids was carried out by flow cytometry and PCR methods using microsatellite markers. After transferring the plants in the greenhouse and in the field serveral self-pollinations and backcrosses were performed. The seed was investigated by gas chromatography to analyze the fatty acid composition. First results which based on the half grain method of somatic hybrids of *B. napus* and *S. alba* indicated an increase of the erucic acid content to approximately 52% at the rapeseed parent up to 65% at the somatic hybrid progeny after backcrossing with a high erucic acid rapeseed line. Additional backcrossings and a selection of the best progenies are planned for maintaining this high erucic acid content.

P-1341

Microspore Culture in Local Indonesian Hot Pepper Accessions. E. DARMO JAYA SUPENA* and J. B. M. Custers. Plant Research International, Wageningen University and Research Centre, P.O Box 16, 6700 AA Wageningen, The Netherlands. E-mail: E.DARMO-JAYA-SUPENA@PLANT.WAG-UR.NL. *Research Center for Biotecnology, Bogor Agricultural University, Bogor, Indonesia.

Aim of our project is to develop and exploit haploid technology for the genetic improvement of hot pepper varieties adapted to cultivation in Indonesia. In a relatively short period we succeeded in establishing so-called shed-microspore culture for hot pepper. Selection of anthers with microspores in the right stage of development appeared to be crucial, while cold pretreatment of the buds (4° C) favored the yield of embryos produced from the microspores. To test the general applicability of the shed-microspore culture for genetically divers Indonesian hot pepper germplasm, 10 varieties or accessions were analyzed, six of big hot pepper and four of the curly type. All genotypes showed to be responsive, bud responsiveness being at least 60%, except for variety 'Typhoon' with 43% bud success. Total embryo yield reached from 5 to 36 embryos per bud in the various genotypes, while 'Typhoon' was again exceptionally low with two embryos per bud only. Overall germination percentage ranged from 14 to 30%. Ploidy analysis revealed 62% of the plants to be haploid, 37% diploid, and only 1% triploid or tetraploid. Extrapolating from our data, we need to incubate anthers from 20–150 buds for the production of 100 microspore-derived plants with hot pepper. This success rate is outperforming by far the results with bell pepper, where the best procedure so far needs incubation of anthers from 1000–1500 buds for getting the same yield of plants.

P-1343

Association of Glufosinate Tolerance and Blackleg Resistance in *Brassica napus* L. MOHAN R. THIAGARAJAH, Gary R. Stringam, Vipan K. Bansal, Delbert F. Degenhardt, and Glen P. Hawkins. Department of Agricultural, Food & Nutritional Science, University of Alberta, Edmonton, Alberta, Canada, T6G 2P5. E-mail: Mohan.Thiagarajah@ualberta.ca

We are developing superior canola breeding lines with herbicide tolerance and resistance to blackleg disease at the University of Alberta, using the microspore-derived doubled haploid (DH) method of breeding. Previous DH studies with the "T177" construct for glufosinate tolerance, and the trait for blackleg resistance, indicated the traits were controlled by two dominant genes, and that there was no association between blackleg resistance and glufosinate tolerance. However, initial studies with the "Rf3" glufosinate tolerant construct suggested a genetic linkage when the two traits entered the cross in repulsion. Segregation of the two genes was consistent with an expected 1:1 segregation ratio for dominant genes, indicating the microspore culture protocol did not impose any gamete pre-selection problems on the system. Subsequent crosses with the two traits in coupling suggested the glufosinate construct was likely linked with blackleg resistance. This finding should enable canola breeders to more effectively develop cultivars having both of these agronomically important traits by selecting for one or the other in a coupling-based breeding program.

P-1344

Antirrhinum majus Microspore Maturation and Transient Transformation In Vitro. A. TOURAEV, I. Barinova, M. Zhexembekova, E. Barsova, S. Lukyanov, and E. Heberle-Bors. Vienna Biocenter, Institute of Microbiology and Genetics, Vienna University, Dr. Bohrgasse 9, A-1030 Vienna, Austria; Shemyakin and Ovchinnikov Institute of Bioorganic Chemistry, Russian Academy of Sciences, 117871 Moscow, Russia, E-mail: Alisher@gem.univie.ac.at

The male gametophyte of higher plants represents an excellent system to study gene regulation, cell fate determination and cellular differentiation in plants because of its relative simplicity compared to the sporophyte and its accessibility for cytological and molecular analysis. Unicellular plant microspores are single haploid cells, which can be isolated in large amounts at a defined developmental stage. Microspores cultured in vitro in a rich medium develop into mature pollen grains, which are fertile upon pollination in vivo. Here we report that isolated Antirrhinum majus microspores when cultured in an optimal medium develop to form mature, fertile pollen. Their development closely resembled that of pollen formed in vivo. Isolated microspores were bombarded with Aquorea victoria Green Fluorescent Protein (GFP), Discosoma Red Fluorescent Protein (dsRFP) and ß-glucuronidase (GUS) reporter genes under the control of various promoters and transient expression was observed throughout pollen development in vitro. Bombarded and not bombarded in vitro matured pollen grains were able to germinate both in vitro and on receptive stigmas and to set seed. The protocol of maturation, transient transformation and germination of Antirrhinum majus pollen in vitro described here provides a valuable tool for basic and applied research.

P-1345

Inhibitory and Stimulatory Factors Regulating Somatic Embryogenesis in Japanese Larch. [1]M. UMEHARA, [2]S. Ogita, [3]H. Sasamoto, [1]C. Eun, [4]Y. Matsubayashi, [4]Y. Sakagami, and [1]H. Kamada. [1]Institute of Biological Science, University of Tsukuba, Tsukuba, Ibaraki, 305–8572 Japan, [2]Research and Education Center for Genetic Information, NAIST, Ikoma, Nara, 630–0101 Japan, [3]Graduate School of Environment and Information Science, Yokonama National University, Hodogaya, Yokohama, 240–8501 Japan, and [4]Graduate School of Bioagricultural Science, Nagoya University, Chikusa, Nagoya, 464–8601 Japan. E-mail: umehara@sakura.cc.tsukuba.ac.jp

It is well known that initial cell density is an important factor for cell proliferation in cell culture. It was reported that the induction of carrot somatic embryogenesis could't be observed at high cell density and stimulated at low cell density. In carrot, it was found that an inhibitor of somatic embryogensis, 4-hydroxybenzyl alcohol (4HBA), and a stimulator, phytosulfokine (PSK) were produced by the culture and accumulated in the medium, and the balance of both factors in the medium determined the success or failure of somatic embryogenesis. Somatic embryogenesis in conifer is also affected by initial cell density. In Japanese larch, when conditioned medium at high cell density (HCM) was added to medium in low cell density culture, somatic embryogenesis was strongly inhibited. Exogenously applied 4HBA didn't act as an inhibitor. It is considered that other chemical substance(s) in HCM is concerned with the inhibition. On the other hand, when conditioned medium at optimum cell density (OCM) was added to medium in low cell density culture, somatic embryogenesis was stimulated. Exogenously applied PSK stimulated somatic embryogenesis, and PSK was found in OCM. These results indicate that unknown inhibitory factor and PSK are produced in larch cell culture and both factors regulate larch somatic embryogenesis.

P-1346

Plant Regeneration from Protoplasts of Cumin (*Cuminum cyminum* Linn.) via Somatic Embryogenesis and Shoot Organogenesis. VIRENDRA MOHAN VERMA and Latika Raisinghani. Agricultural Research Station, Rajasthan Agricultural University, Beechwal, Bikaner-334 006 (Rajasthan) INDIA. E-mail: vmv_vmv@yahoo.co.uk, vmv_vmv@hotmail.com

A study was undertaken to develop a protoplast regeneration system for *Cuminum cyminum* Linn. Protoplasts isolated from hypocotyls and shoot tips of etiolated seedlings were embedded in 1.5% sodium-alginate at a final density 10 x 10^4 protoplast/ml and cultivated in Kao and Michayluk medium containing 0.5 mg/ml of each of 2,4-dichlorophenoxyacetic acid, a-naphthaleneacetic acid and 6-benzylaminopurine. A division frequency of 40% and plating efficiency of 0.5–0.7% were obtained. Six weeks after embedding, protoplast-derived calluses were transferred onto Gelrite solidified Murashige and Skoog's medium containing various growth regulators. Regeneration of plants was achieved via two morphologically distinguishable pathways. A two-step protocol [initially on medium with high auxin concentration (10 mg/l picloram) followed by a culture phase with lowered auxin amount (1 mg/l 2,4-dichlorophenoxyacetic acid)] was used to regenerate somatic embryos, whereas cultivation on medium containing 1.5 mg/l thidiazuron and 0.1 mg/l a-naphthaleneacetic acid resulted in shoot morphogenesis. Mature plants were recovered from both somatic embryos as well as from thidiazuron induced shoots. Half strength MS medium supplemented with auxins (IAA, IBA, IPA and NAA) was used for root induction. Rooted plants were transferred to soil: sand: wood powder: vermiculate (1:1:1:1, v:v:v:v) mixture and acclimatized with 57.5% survival rate. Fully acclimatized plants were planted in the field.

P-1347

Asymmetric Somatic Hybridization for Development of Rapeseed with High Erucic Acid Content. Y. P. WANG[1,2], K. Sonntag[1], B. Hackauf[2], and E. Rudloff[1]. [1]Federal Center for Breeding Research on Cultivated Plants, Institute of Agricultural Crops, Gross Lüsewitz, 18190, GERMANY and [2]College of Life Sciences, Sichuan University, Chengdu 610064, CHINA. Email: wyouping@yahoo.com

PEG-induced asymmetric somatic hybridization between *Brassica napus* and *Crambe abyssinica* was carried out. *C. abyssinica* is an annual cruciferous oil crop with a high content of erucic acid (C22:1) in the seed oil valuable for technical purposes. UV-irradiated mesophyll protoplasts of *C. abyssinica* cv. "Carmen", "Galactica" and "BelAnn" were fused with hypocotyl protoplasts of different genotypes of *B. napus* cv. "Malpus", "11502" and "Erox." Shoot regeneration frequency varied between 5.7% and 20.8% among the different doses of UV-irradiation ranging from 0.05 J/cm^2 to 0.30 J/cm^2. 124 shoots in total were regenerated, of which 20 asymmetric hybrids were obtained and verified by nuclear DNA content and AFLP analysis. AFLP data showed that some of characteristic bands from *C. abyssinica* were present in the hybrids. The investigation into fertility of asymmetric hybrids indicated that the fertility increased with increasing UV-doses ranging from 0.05 J/cm^2 to 0.15 J/cm^2. All of the hybrids were cultured to full maturity and could be fertilized and set seeds after self-pollination or backcrosses with *B. napus*. The analysis of fatty acid composition in their seeds was conducted and found to contain significantly greater amounts of erucic acid (62.6%) than *B. napus*. This study showed that UV-irradiation could be used as a tool to produce asymmetric somatic hybrids and to promote the fertility of the hybrids.

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New Results in Doubled Haploid Production Systems. JENS WEYEN. Saaten-Union Resistenzlabor GmbH, Hovedisser Str. 92, D – 33818 Leopoldshoehe, Germany. E-mail: weyen@saaten-union-labor.de

Doubled haploid lines are increasingly used in breeding systems of monocots and dicots since their first discovery. Andro- and gynogenetic systems are established and doubled haploid varieties are actually registered worldwide. Nevertheless there are still technical problems. Low induction and regeneration rates, genotype dependency, albinism in monocots and low spontaneous chromosome doubling rates are the major obstacles which are responsible for high costs and inefficiency in technologies as anther culture, microspore culture, interspecific hybridization and inducer methods. Variations of medias, growing conditions of donor plants and *in vitro* cultures, *in vitro* medias, pollinator genotypes, stress treatments, antimitotic agents for chemical induced chromosome doubling and much more are possible. We tested different media compositions in anther and microspore culture of spring and winter barley, antimitotic agents in rapeseed microspore culture, different induction medias in wheat anther culture and different colchicination procedures following the wheat corn hybridization technology. It was becoming clear, that glutamin has a beneficial effect on the callus development of barley anther cultures and copper in the concentration of 0,025 mg/l has a beneficial effect on the number of green plants regenerated out of 100 anthers. It was also observed that solidification compounds as sea plaque agarose vs. Agar-Agar and pyridoxin-HCl can have significant effects on induction of barley microspores. The experiments with the herbicide trifluralin as a antimitotic agent in rapeseed microspore culture showed that trifluralin can be a effective, cheap and less toxic alternative to colchicine, although we did not reach as high doubling rates as with colchicine. While the normal colchicination procedure of wheat plants from embryos rescued from cernels induced by corn pollination resulted in a doubling rate of 50%, alternative application methods and stress treatments as cold temperatures and cutting back leaves did not show significant changes in doubling rates. The improvement of production technologies for doubled haploid lines in cereals or rapeseed was tested on a large range of genotypes in barley, wheat and rapeseed. Significant improvements were achieved by variations of medias and antimitotic agents. Stress treatments did not show significant effects on chromosome doubling rates. Generally growing conditions and experience of the laboratory staff is very important. By the combination of different medias, stress treatments and growing conditions we are now able to produce enough fertile doubled haploids from more or less all genotypes which are common in European breeding programmes in barley, wheat and rapeseed.

P-1349

Protocols for Gene Transformation in Strawberry (*Fragaria ananassa* cv. Sweet Charlie). YOUSEF I. AL-DLAIGAN*; Daniel J. Cantliffe, and Harry J. Klee. Horticultural Sciences Department, Univ. of Florida, IFAS, Gainesville, FL 32611-0690. E-mail: dlaigan@ufl.edu, DJC@ufl.edu, hjklee@mail.ifas.ufl.edu

"Sweet Charlie" has many excellent features for both growers and consumers. However, a major disadvantage is soft fruit when ripe. The goal of this work was to establish a protocol(s) for "Sweet Charlie" transformation in order to impart gene(s), which might promote fruit firmness. Explant types, from *in vitro* grown shoots, including leaf-disks, petioles and crown tissues, and shoot-tips from *ex vitro* grown plants were studied. Medium composition, including types and levels of plant growth regulators, such as N^6-benzyladenine (BA), antibiotics, Silwet L-77, aceytosyringone and Drierite, light intensity were altered in an attempt to improve plant regeneration. The ABI strain of *Agrobacterium tumefaciens* carrying a plasmid vector containing a construct harboring the pectin methylesterase (PME) gene, figwort mosaic virus (FMV) as a promoter and kanamycin (k) as a selectable marker were tested. Crown tissues regenerated the highest number of shoots among all explants used. A light intensity of 40–50 μmol. $m^2.s^{-1}$ accelerated the induction and early development of organogenesis. However, lower light intensity (15–20 μmol. $m^2.s^{-1}$) was better for growth and elongation of the developed shoots. Silwet L-77 did not increase the percentage of transformed shoots, and inhibited cell division and was toxic at levels above 100 $\mu l l^{-1}$. Drierite had no effect on the percentage of regenerated shoots. A high level of (BA) $10mgl^{-1}$, (k) $50mgl^{-1}$ and cefotaxime $500mgl^{-1}$ in the initial selection medium, followed by gradual reduction of all three components in the next two subcultures, then increasing the level of k in the third subculture, enhanced regeneration (organogenesis) of putatively transformed shoots. Up to 10% of those shoots survived and continued to grow for more than three weeks, when k levels were increased up to $50mgl^{-1}$.

P-1350

A New Pattern of Somatic Embryogenesis: Embryonic Reconstitution from Cotyledon Lobes of Canola, *Brassica napus* L. PHILIP V. AMMIRATO. Department of Biological Sciences, Barnard College, Columbia University, New York, NY 10027. E-mail: pammirato@barnard.edu

Somatic cells, whether from embryonic, juvenile or flowering plant tissues, typically form adventitious or somatic embryos via a process akin to zygotic embryogenesis. A committed cell (or group of cells) progresses through a series of divisions to establish apical-basal axial polarity, with the apical proembryo emerging from the basal suspensor or suspensor-like cells. Multiple somatic embryos form clusters joined at their base. A new pattern of somatic embryo formation--reconstitution--was observed in small segments of cotyledons removed from young zygotic embryos of canola (*Brassica napus* L.). Excised cotyledonary lobes placed on auxin-containing medium formed hypocotyl-roots on their base within a week. Subsequently, a new opposing cotyledon developed at the juncture of the hypocotyl-root and the lobe. Reconstitution followed clear temporal and spatial parameters: Only cotyledons from the youngest zygotic embryos responded in this fashion and new organ positions were determined by the original apical-basal axial and adaxial-abaxial radial patterns. After transfer to maturation media, a shoot with leaves formed between the regenerated and excised cotyledons, effectively reconstituting complete embryos. New cotyledons showed characteristic anatomy, including the absence of trichomes; hypocotyls developed typical anthocyanin pigmentation in light and then elongated; and roots emerged from the base of the hypocotyl-radicles. Multiple embryos were connected at the bases of the cotyledons. Thus, a small part of the young embryonic cotyledon can reform missing organs and meristems instead of reinitiating embryogenesis de novo.

P-1351

Effects of Long and Brief Induction Pre-treatments on In Vitro Rooting of Pear (*Pyrus communis* L.). MARIA T. F. BARROS, Cláudia I. Hipólito, Ilda C. G. Freire. Instituto Superior de Agronomia, Tapada da Ajuda, 1349–017 Lisboa, Portugal. E-mail: barrosma@isa.utl.pt

Rooting is often a limiting step in micropropagation and may compromise several biotechnology applications. This is particularly so in woody plants and fruit trees are no exception. In a programme of virus elimination from traditional Portuguese cultivars, there was the need to improve the rooting efficiency of selected pear genotypes such as 'Rocha', 'Carapinheira' and 'Pérola'. Micropropagated pear shoots of adult origin had their basal portions immersed in solidified induction medium either with a low auxin level for an extended period (Long Induction Pre-treatment, LIP) or with a high auxin content for a short period (Brief Induction Pre-treatment, BIP). After LIP or BIP, the shoots were transferred to a similar but auxin-free medium (1/2 QL macro-, QL micro-, LS vitamins, 20 g/L sucrose and 7 g/L agar). In a first set of experiments, different LIPs were tested, varying in the duration of the period in darkness and of exposure to IBA. In further assays, one of the LIPs (10 days in 10 mM IBA, the first 5 days in darkness and the remainder under a 16-h photoperiod) was compared to several BIPs (from 30 min to 16 h in medium containing 0.5 or 1.0 mM IBA). LIP revealed a clear distinction in rooting efficiency among the three cultivars. 'Carapinheira' rooted poorly, as opposed to 'Pérola' with ca. 100% rooting. BIPs enhanced rooting of 'Rocha' and 'Carapinheira'. In particular, some BIPs approached the rooting percentage of 'Carapinheira' to that of 'Pérola', eliminating the distinction between 'easy' and 'difficult-to-root' genotypes. The strongest BIPs (12 or 16 h in 1 mM IBA) were detrimental, though, due to an excessive *callus* formation and occasional impaired shoot and/or root development. Experiments involving other important pear cultivars are currently in progress.

P-1352

Somatic Embryogenesis Induction in *Coffea arabica* L. cv. Catuaí. Maria Cristina Simões-Costa[1], MARIA TERESA F. BARROS[2], Carlos José Rodrigues, Jr[1]. [1]Centro de Investigação das Ferrugens do Cafeeiro, Quinta do Marquês, 2784–505 Oeiras, Portugal and [2]Instituto Superior de Agronomia, Tapada da Ajuda, 1349–017 Lisboa, Portugal. [1]E-mail: fbc@mail.telepac.pt

Coffea arabica L. is the most widely cultivated coffee species, accounting for 75% of the world production. Cv. Catuaí resulted from an intraspecific cross of *C. arabica* cvs. Mundo Novo and Caturra, benefiting from the broad adaptability and high yielding capacity of the former and the reduced plant size of the latter. Somatic embryogenesis offers high potential for the large-scale multiplication of elite genotypes. However, nearly all procedures documented in coffee involve very long periods of *in vitro* culture. Also, their efficiency is genotype-dependent and little information is available on cv. Catuaí. Leaf disc explants of Catuaí were incubated in darkness on twelve culture media, to select those leading to an advanced and/or increased embryo yield. *Callus* induction media resulted from a factorial combination of mineral salts (SH, MS and 1/2 MS), vitamins (B_5 and SH) and gelling agents (0.7% agar and 0.25% gelrite). All media included 5 mM 2,4-D and 20 mM BAP. After a 4-week induction period, the explants were transferred to expression media of similar composition but without 2,4-D. No somatic embryos developed on any of the media containing MS or MS/2 salts. Fifteen weeks after initiation, somatic embryos were present in 38.2% of the explants on S11 medium (SH salts and vitamins in agar). This percentage increased to 55.9% two weeks later, when somatic embryos were also observed on 59.4% and 11.9% of the explants on S5 (SH salts + B_5 vitamins in agar) and S12 (SH salts and vitamins in gelrite), respectively. Heart, torpedo and cotyledonary stage embryos were observed as well as secondary embryogenesis. Of these embryo-producing media, S5 gave the highest somatic embryo yield.

P-1353

Agrobacterium-mediated Genetic Transformation of Onion (*Allium cepa* L.) Using Direct Somatic Organogenesis. MANJA-TINA BASTAR, Zlata Luthar, and Borut Bohanec. Centre for Plant Biotechnology and Breeding, Biotechnical Faculty, Jamnikarjeva 101, 1111 Ljubljana, Slovenia. E-mail: borut.bohanec@uni-lj.si

An *Agrobacterium*-mediated DNA delivery system has been developed for onion (*Allium cepa* L.) using organogenic structures formed on ovaries using an established somatic regeneration protocol. Five plasmids and *A. tumefaciens* strains LBA4404 and EHA105 were used to establish an alternative transformation procedure that avoided callus formation. Studies focused on different protocols for cocultivation, selection and detection of optimal reporter and selection genes in onion. Histochemical GUS test and visual detection of GFP protein were used to optimize transformation treatments. Several pretreatment and cocultivation protocols achieving transient expression were studied, the optimal of which was the combination of sonication (10 s) and vacuum treatment (5 min, 35 mm Hg). Among several selection media studied, the optimal results were obtained using phosphinotricin (2.5 mg/l) as selection agent and timentin (150 mg/l) for suppression of bacterial growth. The transgenic nature of individual regenerants, after six months on selection media was confirmed by the polymerase chain reaction using primers for the *uidA* and *bar* genes. Three regenerants were positive for both *bar* and *uidA* genes and three regenerants were positive for the *bar* gene only. In further experiments, wounding with particle bombardment was tested in addition to previously optimized protocol. Such combined treatment gained higher number (3.7%) of regenerants that passed selection. Studies are in progress to confirm stable transformation.

P-1354

Basal Nutrient Medium Affects Axillary Shoot Proliferation and Preconditioning for Adventitious Regeneration of Pears. R. L. BELL[1], C. Srinivasan[1], and D. Lomberk[2]. [1]U.S. Department of Agriculture, Agricultural Research Service, Appalachian Fruit Research Station, Kearneysville, WV 25430 and [2]U.S. Department of Agriculture, Agricultural Research Service, Epcot Science, Lake Buena Vista, FL 32820. E-mail: rbell@afrs.ars.usda.gov

The influence of the basal nutrient composition of plant tissue culture media on axillary shoot proliferation and their preconditioning effect on subsequent adventitious shoot regeneration from leaves was investigated. The goal was to improve both micropropagation and regeneration of 'Bartlett' and 'Beurre Bosc' pear cultivars. Driver-Kuniyuki Walnut (DKW) and Quorin and Lepoivre (QL) basal nutrient media were found to be superior to Murashige and Skoog (MS) and Woody Plant Medium (WPM) for axillary shoot proliferation. Shoots on WPM exhibited some chlorosis. Axillary shoot culture on DKW would be preferred to that on QL due to the production of excessively short, thin shoots on the latter medium. DKW also was superior to QL and MS for production of young expanding leaves for use as explants in adventitious regeneration. Leaf explants derived from shoot proliferation cultures grown on DKW or QL media produced more adventitious shoots than leaf explants from MS in both experiments.

P-1355

A Simple System for Lentil Transformation Using *Agrobacterium tumefaciens* and *In Vitro* Micrografting. KHALID MAHMOOD KHAWAR BHATTI, Serkan Uranbey, Sati Çöçü, Cengiz Sancak, and Sebahattin Özcan. Department of Field Crops, Faculty of Agriculture, University of Ankara, 06110 Dışkapı, Ankara, Turkey.

Lentil is an important food legume crop cultivated widely in Mediterranean region and South Asia and meets protein and mineral requirements of large majority of people inexpensively. It is an under exploited crop and very little studies have been made for its improvement through traditional and modern tissue culture techniques. It was therefore considered necessary to develop a simple protocol for regeneration and thereafter transformation of the plant under *in vitro* conditions. After a considerable study on different genotypes, explants and media, it was found that 0.05 mg/l TDZ had invigorating effect on shoot regeneration from cotyledonary node explants isolated from 3–4 days old seedlings of lentil. These nodes were used to develop a reproducible *Agrobacterium tumefaciens* mediated trans-formation system using GV 2260 p35S GUS-INT strain for transformation in six genotypes. The described procedure resulted in production of large number transgenic shoots. The obtained transgenic shoots failed to root in rooting media containing IBA. Therefore, these transgenic shoots were micrografted on non-transgenic rootstock of variety Kayi 91 to obtain full plant successfully. Most of these grafts were established and transferred to greenhouse. Key words: *Agrobacterium tumefaciens*, lentil transformation, cotyledonary node explant, nptII gene, GUS gene, kanamycin resistance.

P-1356

Gynogenic Haploid Induction in Onion: Role of Genotype and Establishment of Alternative Chromosome Doubling Procedure. Marijana Jakše[1], Michael J. Havey[2], and BORUT BOHANEC[1]. [1]Centre for Plant Biotechnology and Breeding, Biotechnical Faculty, Jamnikarjeva 101, 1111 Ljubljana, Slovenia and [2]USDA-ARS, Dep. of Horticulture, 1575 Linden Drive, University of Wisconsin, Madison, WI 53706. E-mail: borut.bohanec@uni-lj.si

In vitro culture of unpollinated flowers is an efficient procedure to produce doubled haploids (DH) of onion. However several bottlenecks remain which diminish the success rate of DH production, in particular the low gynogenic potential of a majority of lines and cultivars, as well limited chromosome doubling procedures. Our research focused on the inheritance of crosses among responsive and non-responsive lines and the phenotypic correlations for production of haploid regenerants from parental plants and selfed progenies, as well as the establishment of novel chromosome doubling procedures based on treatments of gynogenic embryos immediately after regeneration. The results of these studies showed that crossing of responsive and non-responsive onion lines resulted in increased gynogenic abilities in the hybrid progenies and that there are genetic and environmental components to haploid production. For chromosome doubling experiments, embryos (instead of spliced shoots) were treated with various concentrations of amiprophos-methyl (APM) or oryzalin in liquid under short duration in partial vacuum or longer duration at normal air pressure or on solid media. In general, APM was efficient and less toxic than oryzalin. The addition of DMSO or Triton X100 to treatment solutions did not improve genome doubling. Based on treatments of over 7000 embryos in 2001, we concluded that application of 50 μM APM was superior to 25 or 75 μM, and treatments in liquid medium (1 or 2 days) were superior to the same treatments on solid media.

P-1357

Protocols for Germination and Ex Vitro Establishment From Black Walnut (*Juglans nigra*) Somatic Embryos. MICHAEL J. BOSELA and Charles H. Michler. USDA Forest Service, North Central Research Station, Hardwood Tree Improvement and Regeneration Center, 1159 Forestry Building, Purdue University, West Lafayette, IN 47907–1159. E-mail: mbosela@fnr.purdue.edu

Somatic embryo cultures of *Juglans nigra* were established from June–August 1999 using cotyledon explants from immature zygotic embryos. The cultures have been maintained in the dark on both hormone free media (with DKW and MS nutrient salts) and on MS medium with 2.2 μM 2,4-D. On hormone-free media the secondary embryos produced were direct in origin, while those produced on 2,4-D medium were indirect in origin. For both embryo types, two size classes of embryos were used for germination; i.e. small, 'early' cotyledonary embryos with 0.5–3 mm long cotyledons, and larger cotyledonary embryos with 4–12 mm cotyledons that were produced via culture on hormone free medium for 10–14 days. The embryos were germinated in the light (16 hr photoperiod) on both hormone-free media and derivative media with GA (5 – 10 μM). When the embryos were transferred directly to plates of germination media, germination was only erratically observed (<5% frequency) and the cotyledons frequently resumed growth and proliferated additional embryos. For both sizes and types (direct, indirect) of embryo the cotyledon tissues were typically transparent and presumably deficient in storage reserves that are associated with germination competence as indicated by an opaque (white) embryo condition. To facilitate germination, the embryos were dessicated at 95 to 97% relative humidity over saturated zinc sulfate solutions. The early cotyledonary embryos were dessicated for 7–10 days. Mortality rates were highly variable between experimental runs (0–50%) and the dessication treatment was typically only partly effective for opacity induction. In addition, for most runs 25–50% of the embryos surviving dessication were non-responsive, i.e. they did not germinate and either remained colorless or browned. Root germination frequencies of less than 20% were more typical and root germination was largely confined to the subset of embryos that had converted to a white condition. When larger cotyledonary embryos were employed, the mortality rates were lower (0–20%) and opacity was induced at frequencies of nearly 100%, however, dessication periods of 3–4 wks were required for efficient germination and cotyledon greening. For embryos of direct origin, root germination frequencies of at least 60% were typical and for some runs, root germination was observed at frequencies of up to 80%. When embryos of indirect origin were employed, swelling of the embryo axis was frequently observed instead of root elongation, however, GA inclusion facilitated root germination. For both embryo types, concurrent root and shoot germination were rarely observed. Maximal epicotyl germination frequencies ranged from 10–15% on a per run basis. Furthermore, in most cases the degree of epicotyl development was limited, i.e. only tiny foliage leaves to 3–4 mm were produced, and the embryos were not able to survive transplanting. Vigorous epicotyl germination responses (leaves to 10 or more mm in size and stem elongation) were observed only erratically, however the embryos could be successfully transplanted. The epicotyls of the shoots appeared viable since they could be induced to elongate following excision and transfer to media with zeatin (5–15 uM). Media types that could be used to facilitate epicotyl growth prior to transplanting without adversely affected root health are currently being evaluated.

P-1358

Effect of 2,4-Dichlorophenoxyacetic Acid and Osmotic Stresses on the Establishement of Cell Suspension and Plant Regeneration in Wheat (*Triticum aestivum* L.). ROSA MARIA BRITO and A. Pellegrineschi. Applied Biotechnology Center, CIMMYT, Mexico. E-mail:rosmary68@hotmail.com

Immature embryos from a high regeneration line of spring wheat (*Triticum aestivum* L.) Bobwhite SH 98 26 and durum wheat (*Triticum turgidum* var. *durum* L.) were cultured in E3 callus induction media. (Murashige-Skoog (MS) medium, 2.5 mg.l-1 2,4-dichlorophenoxyacetic acid, 2% sucrose and 0.9% bactoagar). Embryoids like structures and calli were transferred to a E3 liquid suspension medium supplemented with again with 2,4-D and NaCl, for each in vitro treatment. Incubation with 2.5 mg/l 2,4-D for 45 days produced callus and plant regeneration in Bobwhite but lower callus yield and plant regeneration in Mexicali, indicative of 2,4-D only was not sufficient for callus induction and plant regeneration in this variety. Callus yield and regeneration frequencies were higher in Maxicali, adding NaCl in the initiation phase. The extended presence of NaCl in the medium beyond the initiation phase was detrimental to plant regeneration. Thus optimal callus induction and plant regeneration could be obtained in Mexicali through manipulating the NaCl concentration and the duration of its presence in the induction medium.

P-1359

Culture Medium Requirements for the In Vitro Establishment of Shoot-tip Cultures of the Portuguese Apple Cultivar 'Bravo de Esmolfe'. MARIA IRENE CANDEIAS[1], Miguel R. T. Mascarenhas[1], Maria Teresa F. Barros[2]. [1]Estação Agronómica Nacional, 2784–505 Oeiras, Portugal. [2]Instituto Superior de Agronomia, Lisboa, Portugal. [1]E-mail: irene_candeias@clix.pt

'Bravo de Esmolfe' is a traditional Portuguese apple with unique organoleptic characteristics. In a sanitary evaluation, all clones (>150) were positive for at least one viral disease. Because this is a highly valued cultivar, a program aiming at virus elimination through shoot-tip culture is in progress. Preliminary assays using culture media suitable for shoot-tip culture of other apple cultivars were unsucessful. In particular, sucrose (20 or 30 gl^{-1}) in MS medium was inhibitory of shoot formation. Although sorbitol (20 gl^{-1}) could replace sucrose with success, the shoots obtained were abnormal. To improve these results, experiments were conducted using bud-tips (»1 mm) from different clones. Modified MS (1/2 macro-), QL, WPM and DKW media were compared, all including 0.5 mgl^{-1} BAP and 20 gl^{-1} sorbitol. Medium did not influence shoot formation but had a highly significant effect on shoot morphology and *callus* formation. QL led to the significantly highest frequency of normal shoots (100%) without *callus* outgrowth. Using this superior medium, BAP concentration was assayed. The lowest (0.1 mgl^{-1}) resulted in a highly significant reduction in shoot formation. No significant differences were found between 0.5 and 1.0 mgl^{-1} BAP, both for shoot formation and development, but shoots on 1.0 mgl^{-1} were frequently affected by chlorosis. The effect of sugar in QL medium plus 0.5 mgl^{-1} BAP, was also evaluated. Shoot formation was significantly lower in the presence of glucose (20 gl^{-1}). The increase in sorbitol from 20 to 30 gl^{-1} did not affect shoot formation but significantly reduced shoot development (fresh weight, no. of leaves). Addition of GA_3 (0.1 mgl^{-1}) or IBA (0.05 mgl^{-1}) did not result in any further improvements. No significant clonal effects were noticed.

P-1360

In Vitro Conservation of *Colocasia esculenta* (L.) Schott Germplasm. Isabel R. Moura[1] & MARIA F. CARNEIRO[2]. [1]Jardim-Museu Agrícola Tropical/IICT, Largo dos Jerónimos, 1400–209 Lisboa, Portugal; [2]Centro de Investigação das Ferrugens do Cafeeiro/IICT, Quinta do Marquês, 2784–504 Oeiras, Portugal. E-mail: jmust@iict.pt

Colocasia esculenta (L.) Schott (taro), is largely cultivated in tropical, subtropical and some temperate regions, (such as Azores islands), for it's edible tuberous rhizomes. As *C. esculenta* is vegetatively propagated, the germplasm preservation requires the maintenance of field repositories, with inherent costs and land requirements, and risks of losses. A project aiming to establish an *in vitro* germplasm collection of the referred species is being developed at the Jardim Museu Agrícola Tropical. As a first step, a micropropagation technique using vegetative apices as initial explants was optimized. The best multiplication rates were obtained on media supplemented with 2 mgl^{-1} BAP and 0,1 mgl^{-1} NAA. In a second step, with the objective of evaluating the best conditions for *in vitro* conservation by slow growth induction, several parameters were tested: reduction of the level of mineral salts (MS and MS/2); reduction of the carbon source concentration (sucrose 30–15 mgl^{-1}); use of manitol (15 mgl^{-1}) and addition of a growth inhibitor (ABA 1–10 mgl^{-1}). After six months in culture, the decrease in sucrose level, with or without manitol, showed little effect on plant growth, but the reduction of the mineral salts level to a half and the inclusion of ABA at 10 mgl^{-1} reduced greatly plant growth and apparently did not affect the explants survival. Results obtained indicate that modification of the composition of the culture medium allows the medium term conservation of *Colocasia esculenta* with considerable reduction of subculture intervals.

P-1361

Ploidy-dependent Reproduction Pathways in Saint Johns Wort (*Hypericum perforatum* L.). E. CELLAROVA and J. Koperdakova. Department of Experimental Botany and Genetics, Faculty of Science, P. J. Safarik University, Manesova 23, 041 54 Kosice, Slovakia. E-mail: cellarov@kosice.upjs.sk

Seed formation in natural populations of *Hypericum perforatum*, a medicinal plant with considerable pharmaceutical effects, is highly polymorphic as proved by flow cytometric seed screen (FCSS). This work is aimed at the screening of reproduction pathways in somaclonal families of *in vitro* regenerated plants and three subsequent generation of progenies of different ploidy. In vitro differentiated somaclones of tetraploid origin showed an extensive variation in the chromosome number. The analyses of seed samples of diploids by FCSS revealed that 93% reproduce sexually forming 2x(3x) seeds, i.e. B_{II} hybrids. The analyses of the seed samples of triploids, tetraploids, pentaploids and hexaploids revealed co-existence of several different modes of reproduction. The tetraploids were prevalently facultative apomicts producing B_{III} hybrids, parthenogenetic and aneuploid seeds and twin embryos. While the plants of different ploidy were able to produce B_{II} hybrids, production of B_{III} hybrids, parthenogenetic and apomictic seeds seemed to be closely associated with ploidy. In several diploid lines only production of B_{II} hybrids has been found. These results contribute to the knowledge that apomixis is not effectively expressed at diploid level.

P-1362

Comparison of Micropropagation Methods for *Typnonium flagelliforme* (lodd.) Blume, a Medicinal Plant with Anti-cancer Potential. CHAN LAI KENG, W. Y. Koh, T. S. Su, and Richard E. Litz. Tropical Research and Education Center, Institute of Food and Agriculture Science, University of Florida, 18905 SW 280 Street, Homestead, FL 33031. Email: lkchan@usm.my

Three different micropropagation methods for multiple shoot formation of *Typonium flagelliforme* (Lodd.) Blume were investigated: 1) semi solid medium; 2) liquid culture with continuous agitation on an orbital shaker; and 3) liquid culture with continuous aeration. After 21 days of culture, the number of shoots regenerated from each explant was found to be statically different among the different methods. Shoot tips produced 22.2 shoots per explant within 21 days when cultured on semi solid medium consisting of MS (Murashige and Skoog, 1962) salts and organics, supplemented with 0.3 mg/L N^6-benzyladenine (BA), 0.5 mg/L indole-3-butyric acid (IBA), 30g/L sucrose, and 7.5 g/L agar (Difco-bacto). The liquid culture medium was MS supplemented with 0.3 mg/L BA, 0.5 mg/L IBA, and 40 g/L sucrose. Shoot tips cultured in liquid medium with continuous agitation on an orbital shaker produced 32 shoots per explant within 21 days. With continuous aeration at 15 ± 2 ml/min, the number of multiple shoots regenerated was 44 shoots per explant with 21 days; however, shoots in liquid medium were hyperhydric. The temporary immersion method is being investigated to eliminate hyperhydricity and as a potential mass propagation technique

P-1363

A Novel and Efficient Method for Genetic Engineering of Plants. QIMIN CHAO, Christine Sullivan, John Getz, Kathryn Gleason, and Luigi Grasso. Morphotek Inc., 3624 Market Street, Suite 508, Philadelphia, PA 19104. Email: qchao@morphotek.com

Historically two approaches have been used to create new traits in plants. One approach involves transferring a single gene at a time into a host plant and is of limited use in the case where a complex trait is sought that is multigenic. A second approach has been through conventional chemical mutagenesis of seeds to generate mutants whereby the trait is selected following batch mutagenesis. This approach is limited by the substrate specificity that different chemical mutagens have for DNA and is generally a toxic process that leads to reduced plant viability. In order to enhance the genetic diversity of a host plant, we developed a novel gene altering platform technology, called *morphogenics*, that can alter a host organism's genome *in vivo*, yielding offspring with a wide array of novel phenotypes. *Morphogenics* has been successfully applied to a variety of organisms, including plants such as *Arabidopsis thaliana*, to produce a number of offspring exhibiting a wide array of phenotypes, such as albinism, dwarfism and ethylene insensitivity. Genome analysis of morphogenic-derived plants has demonstrated the ability of this process to produce a wide spectrum of genetic changes, including genome-wide missense and insertion/deletion alterations. Other advantages observed in morphogenic-exposed plants are: 1) a lack of toxicity to the host; 2) a high-frequency of genetic evolution; and 3) a perpetual process of genetic evolution; all attributes that are important for maximizing genetic diversity and increasing the generation of plants exhibiting novel phenotypes. Moreover, significantly fewer seeds are required for screening with morphogenic derived plants in order to identify offspring with novel output trait(s) in contrast to chemical mutagenesis (3,000 as compared to 100,000 respectively) due to the robust nature of the morphogenic process. This greatly simplifies the primary screen to isolate complex traits with enhanced, novel phenotypes in crop plants.

P-1364

The Role of Micropropagation in Foliage Plant Production: Review and Forecast. JIANJUN CHEN and Richard J. Henny. University of Florida, IFAS, Mid-Florida Research and Education Center, 2725 Binion Road, Apopka, FL 32703. E-mail: JJCHEN@MAIL.IFAS.UFL.EDU

Most foliage plants were traditionally propagated asexually by cuttings or divisions, which is not only time consuming, but also requires much greenhouse space to maintain stock plants and has potential to spread diseases. Since the initiation of foliage plant micropropagation during the mid-1970s, more than 50% of the 100 major foliage plant genera are now propagated in vitro. Florida has led the nation with annual production of liners (starting materials) at more than 50 million in the worldwide market. In vitro liner production helps to eliminate systemic diseases in starting materials, dramatically reduces greenhouse spaces required for maintaining stock plants, and provides growers liners on a year round schedule. In addition, micropropagation has become a prosperous avenue to obtain new cultivars through the selection of somaclonal variants. More than 400 cultivars have been selected and released. Through micropropagation, new cultivars reach numbers to become commercially available in 2 to 3 years compared to the 5 to 10 years needed for traditional propagation methods. According to the USDA National Agricultural Statistics Service, the national wholesale value of foliage plants increased from $48.5 million in 1975 to $574 million in 2000. The importance of micropropagation in evolving foliage plant production and the growth of the foliage plant industry will be discussed.

P-1365

Development of A High Throughput Transformation System in Flax. YURONG CHEN and Paul Dribnenki. Linola™ Breeding Program, Agricore United, Unit 100–101, Route 100, Morden, Manitoba, Canada R6M 1Y5. E-mail: ychen@agricoreunited.com

Flax (*Linum usitatissimum* L.) is the second most important oil crop in Canada, a traditional source of high quality natural fibers in Asia and Europe, and also used as nutraceuticals for human and protein source for animal rations. Linola™ is a modified form of flax that contains high quality edible oil with high polyunsaturated and low saturated fat. The properties of small genome size, strict self-pollination and in vitro totipotency would make flax as a favorable model system for functional genomic studies, an ideal vehicle for the commercialization of genetically modified products, and an easy target for the improvement of agronomic traits through genetic engineering. A highly efficient Agrobacterium-mediated transformation system was developed using anther culture-derived callus as the ex-plant. Transgenic shoots were regenerated from the transformed callus within six weeks after co-cultivation. In this system, the transformation efficiency defined as the number of independent transgenic plants/per anther culture-derived callus reached 66.0%. This transformation efficiency was approximately four times higher than the best reported transformation efficiency using hypocotyl as the ex-plant. The escape frequency in this system was only about one third of that of using hypocotyl as the ex-plant. This high throughput transformation system has been successfully demonstrated using hygromycin as the selective agent to select for a reporter gene (GUS) or agronomically important genes (flax rust resistance genes).

P-1366

In Vitro Micropropagation of *Exacum*. SIVA CHENNAREDDY and Andrew Riseman. UBC Botanical Garden and Centre for Horticulture. University of British Columbia, Vancouver, BC, V6T 1Z4. Canada, E-mail: sivachennareddy@hotmail.com

In support of a new crop development breeding program, the micropropagation of interspecific hybrids of *Exacum*, derived from species native to Sri Lanka, are reported. Multiple shoot formation was obtained from nodal explants excised from plants growing in the greenhouse on a basal MS medium supplemented with 0.1 mg/L alpha-naphthaleneacetic acid (NAA) and either 1–2 mg of benzyl adenine (BA) or 2-isopentenyladenine (2iP), on solid media. Shoot enlargement and development began after 2 weeks in culture in all treatments regardless of the hormone combination. Shoots were sub-cultured every 4 weeks. In the first sub-culture, an increase in the multiplication rate was observed with increasing concentrations of either 2iP or BA. In treatments with BA, callus growth at the base of the explant was significant, while no callus was formed in the 2iP treatment. In addition, shoots produced in the BA treatment were thin and weak while shoots produced in the 2iP treatment were thick and strong. Significant genotypic x media interactions were observed for multiplication rate. In genotypes E-37 and E-32, the multiplication rate increased with increasing concentration of 2iP, with a maximum rate of 3.6 observed at 2 mg/L 2iP. However, in genotypes E-6 and E-23, the multiplication rate decreased with increasing concentrations of 2iP with a maximum rate of 3.3 observed with only 1 mg/L 2iP. In BA treatments, a concentration of 2 mg/L produced maximum multiplication rates of 4.3 and 2.9 for genotypes E-6 and E-37, respectively. However, BA concentration of 1 mg/L produced maximum multiplication rates of 3.7 and 1.9 for genotypes E-23 and E-32, respectively. After the 3rd sub-culture, *in vitro* rhizogenesis was induced on the shoots of genotypes E-37 and E-6. Hormone supplements of 1 mg/L NAA plus 0.1 mg/L 2iP resulted in approximately 85% rooting in either genotype, while 1 mg/L NAA plus 0.1 mg kinetin resulted in approximately 62% rooting in either genotype. Rooting percentage was significantly reduced with IBA treatments in both genotypes. Hormone supplement of 1 mg/L IBA plus 0.1 mg 2iP produced approximately 25% rooting, while 1 mg/L IBA plus 0.1 mg/L kinetin, produced only 10% rooting. This research demonstrates micropropagation of interspecific *Exacum* is possible and that acceptable multiplication percentages are possible.

P-1367

Gene Transfer by *Agrobacterium tumefaciens* to Androgenic Embryos of Winter Oilseed Rape (*Brassica napus* L.). T. CEGIELSKA-TARAS, T. Pniewski*, L. Szała, K. Miedzińska*. Plant Breeding and Acclimatization Institute, 60–479 Poznań, Strzeszyńska 36, Poland and *Institute of Bioorganic Chemistry, 61–704 Poznań, Noskowskiego 12/14, Poland. E-mail: tceg@nico.ihar.poznan.pl

The efficiency of transformation is influenced by many factors, oilseed rape genotype, type of explant, conditions for plant regeneration and selection, and DNA construct. Microspore-derived embryos (MDEs) of homozygous line DH-O120 and cultivar Bor were inoculated and cocultivated with *Agrobacterium tumefaciens* strains LB4404 and EHA105 and contain binary plasmid pP35SGIB with GUS gene with intron under 35SRNACaMV promoter and *bar* gene as a selectable marker. Cotyledons and hypocotyls were puncture with a sawing needle dipped in *A. tumefaciens* suspension for 2 hours. The explants were cocultivated with *A. tumefaciens* for two days and transferred to medium with antibiotics and 10 mg/l herbicide Basta as selective agent for 4 weeks. After that period the green explants were transferred to shoot induction medium with antibiotics and selective agent Shoots remained green on this medium were considered as transformants and were placed in root induction medium and then in the soil for further development. PCR analysis was done to detect the presence of the *bar* gene in the regenerated plants and confirm that transformation was effective. In putative transgenic plants it indicated amplification of a 516 bp fragment of the *bar* gene. The first results of PCR analysis showed that 30% of plants regenerated from transformed microspore-derived embryos were transformants. The progeny of the plants, seeds of T_1 generation, will be tested for resistance to herbicide Basta.

P-1368

Competence for In Vitro Bulblet Regeneration Among Eight *Lilium* Genotypes. M. E. COMPTON and L. R. Canon. School of Agriculture, University of Wisconsin-Platteville, 1 University Plaza, Platteville, WI 53818. E-mail: COMPTON@UWPLATT.EDU

Competence for *in vitro* bulblet regeneration was investigated for eight *Lilium* genotypes (*L. tigrinum* 'Orange Star,' *L. tigrinum*, *L. speciosum* 'Rubrum', *L. sp.* 'Stargazer,' *L. sp.* 'Stone,' *L. sp.* 'Lovely Girl,' *L. tenuifolium*, and *L. citronella*). Small (5mm x 5 mm) bulb scale explants were cultured on lily bulblet regeneration medium [modified MS (1962) with per liter 0.825 g NH_4NO_3, 0.170g KH_2PO_4, 30g sucrose, 100 mg myo-inositol, 0.4 mg thiamine·HCl, 2 ml PPM, 80 mg adenine sulfate, 16 microM naphthaleneacetic acid and 4.5g AgarGel at pH 5.7] for 8 weeks before transfer to peat moss and 4 weeks refrigeration at 5C. Refrigerated bulblets were transferred to the greenhouse for sprouting. All genotypes tested produced bulblets *in vitro*. However, there was an interaction for bulblet production between genotypes and the date when bulbs were collected. Explants from bulbs collected in early October, when plant stems were vigorous, produced about 1.3-fold more (2.1) bulblets than those collected when plants were dormant in late October (1.7). However, the percentage of explants that produced bulblets was similar between sampling dates (~67%). When comparing *in vitro* and *in vivo* bulblet production, about twice as many bulblets were produced by explants *in vitro* (1.7) compared to halved scales incubated in the greenhouse (0.8). In addition, more *in vitro* explants (65%) produced bulblets compared to halved scales in the greenhouse (47%). This study demonstrates that a wide range of Lilium genotypes are competent for *in vitro* bulblet formation and that bulblet formation *in vitro* is more efficient than *in vivo* techniques.

P-1369

Shoot Formation from Vegetative Shoot Apices in Walnut and Relationship Between the Organogenic Response and Cytokinin Accumulation. E. Caboni[1], S. D'Angeli[2], A. Chiapetta[3], A. M. Innocenti[3], and C. DAMIANO[1]. [1]Istituto Sperimentale per la Frutticoltura, Ciampino Aerporto I-00040 Roma, Italy; [2]Dipartimento di Biologia Vegetale, Università di Roma "La Sapienza" P.le Aldo Moro 5, I-00185 Roma, Italy; and [3]Università degli Studi della Calabria, Dipartimento de Ecologia, Laboratorio di Botanica, Cubo 6B, 87030 Rende, Cosenza, Italy. Email: ist.pzopag@mclink.it

Since the conventional breeding is particularly difficult and long term requiring in walnut (*Juglans regia* L.), a wider availability of regeneration systems to be used for application of gene transfer methods could be desirable. In this work, shoot formation was obtained from callus produced from main vegetative apices of *in vitro* grown shoots of a seedling of an Italian cultivar, Sorrento. The explants were maintained on a LP medium containing 8.8 μM BA and 1.0 μM NAA for 30 days in darkness and then transferred to an auxin-free medium and to the light. More than 90% of explants produced 4–5 shoots. The histological studies are now in progress to determine whether all the new vegetative buds originated from the callus. *In situ* localization analyses of cytokinins were also performed using polyclonal rabbit antibodies which selectively immunolabel in plant tissues only free base Zeatin (Z) or isopenteniladenine (Ip). iP immunolabelling on sections showed an unspecific, widespread localization which could be explained with the role of precursor of the other endogenous cytokinins of this molecule. On the contrary, antibodies against Z revealed a greater specificity: only well delimited areas were marked and it was evident that in the shoot formation process Z localization plays also in walnut a role in cell division leading to callus formation and in the differentiation of vegetative buds as already showed in apple and pear.

P-1370

A Novel Meristem Regeneration System for Wheat. YINGHUI DAN, Tishu Cai, and Joyce E. Fry. Monsanto Company, GG4B, 700 Chesterfield Parkway North, St. Louis, MO 63198. Email: yinghui.dan@monsanto.com

A highly efficient and genotype-flexible organogenic regeneration system has been developed using meristem explants. Bobwhite and three elite lines, which ranked from the best to the worst for regeneration ability based on a previous study of 41 elite lines using immature embryo explants, were investigated. For the wheat elite lines and Bobwhite, 61.1 to 98.1% of explants produced 20 to 140 buds or shoots per explant, respectively. An average of 20 to 48 elongated shoots per explant was obtained, and 92.1% of shoots produced roots. Plant regeneration took 10–11 weeks. The three elite lines produced a similar response for organogenic regeneration as Bobwhite, indicating that this regeneration system will be useful for many genotypes. The majority of current wheat regeneration methods depends on immature embryo explants derived from mature plants grown in the greenhouse or growth chamber. This simple and reliable technology has led to the development of a highly efficient regeneration system in which the explant tissue source is derived from seedlings or mature seeds.

P-1371

Somatic Embryogenesis From Cell Suspensions of Banana Clones Resistant and Susceptible to Sigatoka Disease. EVA DE GARCIA, M. I. Arteaga, and T. E. Vargas. Laboratorio de Biotecnología Vegetal, Instituto de Biología Experimental, Facultad de Ciencias, Universidad Central de Venezuela, Apartado 47114, 1040 Caracas, Venezuela. E-mail: egarcia@reacciun.ve

Banana are perennials herbaceous plants that belong to *Musaceae* family. They show ploidy and sterility, which is not helpful to develop new varieties using conventional genetic improvement methods. This led to the evolvement of new strategies within tissue culture techniques and molecular biotechnology in order to solve the difficulties that arose when trying to improve this culture genetically. Among these strategies we cite somatic embryogenesis and cellular suspensions, which have made possible to obtain "in vitro" banana plants, establishing a regeneration process from a single vegetative cell, that allows the use of different biotechnology techniques to improve the crop and perform specific cellular essays. The present work is based on the development of embryogenic cellular suspensions in two different banana clones with a distinct susceptibility degree to Yellow and Black Sigatoka. Both are common diseases in banana plants caused by fungus, and its incidence is spread word-wide in banana plantations. The disease indirectly causes early death of great quantities of foliar surface, which leads to the reduction of photosynthetic functions and the alteration of the normal physiological maturity. One clone is the somaclonal variant CIEN-BTA-03 which was obtained in the Vegetal Biotechnology Laboratory group (UCV, Venezuela). This somaclone has demonstrated on field essays to be resistant to Yellow Sigatoka; besides, field evaluation demonstrates that it also shows some resistance to Black Sigatoka. The other is the clone Williams from the Cavendish subgroup (triploid acuminate (AAA), which is a crop susceptible to Sigatoka. Three induction media for calli formation were tested on both clones. Abundant white embryogenic calli were formed after 45 days of the initiation of the culture in medium MS, supplemented with 1mg/l 2,4-D, 1mg/l zeatin, vitamins and 100 mg/l ascorbic acid. Cellular suspension from those calli were established after 30 days in MS media with 2,4-D, zeatin, vitamins and ascorbic acid or MS media with 2,4-D, Morel vitamins and cysteine.The formation of embryos from both clones was observed two months after cell suspension initiation. The regeneration of plants was achieved in media depleted of 2,4-D.

P-1372

Establishment of an Efficient System of Somatic Embryogenesis from Cellular Suspension of *Solanum tuberosum* L. CV. Dèsirèe Biochemical Markers Related with the Processes. EVA DE GARCIA, T. E. Vargas, and M. Oropeza. Laboratorio de Biotecnología Vegetal, Instituto de Biología Experimental, Facultad de Ciencias, Universidad Central de Venezuela, Apartado 47114, 1040 Caracas, Venezuela. E-mail: egarcia@reacciun.ve

Somatic embryogenesis of *Solanum tuberosum* L. cv. Dèsirèe from mature tissue, was reported for the first time by García and Martínez (1995). They got the somatic embryos from stem nodal sections growing in solid MS media supplemented with vitamins, yeast extract and 2,4-D (dichlorophenoxiacetic acid), sucrose and gelrite. The present work improves the results obtained in that research by means of the establishment of an efficient system of somatic embryogenesis from cellular suspension of stem internode sections of the same cultivar. Besides, the process is biochemically characterized in order to identify biochemical markers related to the different stages of the development of the embryos. To induce calli formation, stem internodes were grown in solid Ms media, supplemented with 0,4 mg/l thiamin, 0,5 mg/l pyridoxine, 100 mg/l myo-inositol, 0,5 mg/l nicotinic acid, 2mg/l glycine, 25 g/l sucrose, 4 mg/l 2,4-D and 2 g/l gelrite. Tissues were incubated at 25 C in dark condition. The establishment of the cellular suspension and the development of the embryos were achieved, when calli formed in the internode sections were cultured in liquid in MS media, supplemented with the same vitamins, 0,5 mg/l Kinetin, and 5 mg/l 2,4-D. Embryos formation was induced in media devoid of 2,4-D, supplemented with 0,5 mg/l of Zeatin, and 100 ml/l coconut milk. This system is very efficient in relation to the embryo production, close to 600 embryos were differentiated in 50 ml of media. SDS-PAGE electrophoretic patterns of the extracellular proteins produced in four different phases of the process of somatic embryogenesis were analyzed. It was found a similarity between the electrophoretic patterns of extracellular proteins produced in the Phase B (cellular suspension with free dividing embryogenic cells), the Phase C (cellular suspension with aggregate embryogenic cells and globular embryos), and the Phase D (cellular suspension with somatic embryos in different stages of development). In all these phases cells were grown in media without 2,4-D. On the contrary, proteins produced in Phase A, which is formed by free and aggregate cells, growing in media with 2,4-D, presented a quite different electrophoretic pattern. It was possible to determine some proteins markers associated to different phases of the somatic embryogenic process.

P-1373

Biolistic Transformation of Potato (*Solanum tuberosum*). Thanh T. Nguyen, Greg Nugent, and PHILIP J. DIX. Biology Department, National University of Ireland Maynooth, Maynooth, Co. Kildare, Ireland. Email: thanh.n.thi@may.ie

An efficient regeneration system in potato, suitable for biolistic transformation, is described. Leaf explants of ten potato cultivars were tested on a range of culture media. Shoot regeneration was most successful on a regime involving incubation of callus induction medium, containing 5 mg/L NAA and 0.1 mg/L BA for 7–10 days, followed by transfer to a shoot induction medium with 2.5 mg/L ZR, 0.2 mg/L NAA and 0.2 mg/L GA_3. The highest shoot regeneration rate was for variety Desiree for which 100% of explants yielded adventitious shoots. The lowest shoot regeneration frequency was for Carrick and Rooster. This protocol was used for biolistic transformation of Desiree. Transient and stable transformation of potato was carried out with plasmid pGUS-HYG. This plasmid contains a chimeric *uid*A reporter gene, that encodes β-glucuronidase (GUS), and a selectable hygromicin phosphotransferase gene (*hpt*). The plasmid pGUS-HYG was bombarded into leaf explants of the variety Desiree. GUS-expressing transgenic shoots, which rooted in media containing 20 mg/L hygromicin, were obtained from plates bombarded with pGUS-HYG. This protocol has been further developed for stable transformation of chloroplasts in potato.

P-1374

Can TAIO (Targeting Agrobateries Into Ovaries) be a New Approach for Crop Transformation? SOPHIE DUCERF. Aventis CropScience, 14/20 rue Pierre Baizet BP 9163, 69263 Lyon, Cedex 09 France. Email: sophie.ducerf@aventis.com

Arabidopsis thaliana transformation via *Agrobacterium tumefaciens* is an interesting and academic example of *in planta* transformation. A simple infiltration of Arabidopsis flowers with an Agrobacterium suspension (Bechtold et al., 1993) allows us to obtain a good number of transformants without a tissue culture step. The selection is done directly on the progeny with an appropriate selectable marker. Ye et al. (1999) showed that the Agrobacterium transformation target is the ovule and Bechtold et al. (2000) that the chromosome set of the female gametophyte is the main target for the T-DNA.Furthermore, *in planta* Agrobacterium transformation is an interesting tool because of the simple integration profile of the transgene in the plant genome compared to naked DNA technologies.We wondered if such a technology could also be developed for crops. We have evaluated an *in planta* approach for soybean called TAIO (Targeting Agrobacteries Into Ovaries).The idea is to deliver an Agrobacterium suspension *in planta* directly into the target organ ie the ovaries, passing through the physical barriers (membranes). Agrobacterium is then directly in contact with the ovules to transfer the T-DNA. In practice we used a micro-injection system involving the injection of the bacterium suspension into the ovaries via a micro-capillary connected to a micro-injector. This was done using a binocular microscope to control the dissection of the flower and to target the ovaries. In this poster we discuss the possibility of using this technology to routinely transform soybean.

P-1375

Transgenic Allium Fingerprinting for Quality Control and Breeding. C. C. EADY, S. Davis, A. Catanach, F. Kenel. Crop & Food Research Ltd, PO Box 4704, Christchurch, New Zealand. E-mail: eadyc@crop.cri.nz

Allium transformation is still in its infancy. However, lines with potentially useful agronomic traits have been produced and these are currently being developed further with the ultimate goal being commercialization. To achieve this it will be necessary to simply and rapidly trace the subsequent lines that contain a particular insert for breeding and quality control purposes. Tail—PCR methods have been developed to detect specific T-DNA inserts in particular transgenic leek and onion lines. Sequenced TAIL—PCR products from these lines are being used to develop primers that flank the T-DNA integration site at the left and right border. We are currently determining whether these can be used accurately as diagnostic tests to confirm the presence or absence of a particular transgene within a particular plant. The production of a simple PCR technique to detect presence / absence and hemizygous vs homozygous condition is vital for effieicent quality control and for the rapid production of breeding lines in this commodity crop.

P-1376

Direct Regeneration of Transgenic Sugarcane Following Microprojectile Transformation of Regenerable Cells in Thin Transverse Section Explants. A. R. ELLIOTT*, R. J. Geijskes**, P. Lakshmanan**, M. G. McKeon**, L. F. Wang**, N. Berding***, C. P. L. Grof*, and G. R. Smith**. *CSIRO Plant Industry, Long Pocket Laboratories, Brisbane, Qld 4068, Australia; **David North Plant Research Centre, Bureau of Sugar Experiment Stations, Indooroopilly, Qld 4068, Australia; and ***Meringa Sugar Experiment Station, BSES, Gordonvale, Qld 4865, Australia. Email: ADRIAN.ELLIOTT@PI.CSIRO.AU

An efficient transformation technique has been developed to directly regenerate fully transgenic sugarcane plants produced by microprojectile bombardment of thin section explants. The green fluorescent protein (GFP) reporter system was used to identify the presence of transformed cells on the surface of the explant and in the regenerating shoots, plants and tillers. The pattern of GFP expression also confirmed that the directly regenerated plants and tillers were homogeneously transformed. Both leaf roll tissue and developing inflorescence provided explants amendable for transformation. The cells that directly regenerated into plants on the surface of the explant were identified using the GFP system. This permitted work to optimise the transformation procedure to proceed. Identifying the precursor cells during regeneration, optimising the transformation frequency of these cells and the timing of application of antibiotic selection after regeneration were critical to the successful production of transgenic plants. The technique was originally developed using sugarcane and is currently being extended to cereals such as wheat which can also be directly regenerated from thin transverse section explants.

P-1377

Agrobacterium tumefaciens-mediated Genetic Transformation of Barley (*Hordeum vulgare*) and Wheat (*Triticum aestivum*). C.-L. Zhang, X.-Y. Ke, A. McCormac, X. Zhang, S. M. Daskolova, M. R. Fowler, N. W. Scott, A. Slater, and MALCOLM C. ELLIOTT. The Norman Borlaug Institute, De Montfort University, Scraptoft, Leicester, LE7 9SU, UK. E-mail: melliott@dmu.ac.uk

An inexpensive routine, high frequency, genotype-independant transformation system is required to facilitate both fundamental research and crop improvement of barley and wheat. *Agrobacterium*-mediated DNA delivery promises to provide such a system. Highly efficient regeneration systems have been established from immature embryos or microspores of several commercial barley and wheat cultivars. Co-cultivation procedures were optimised by assessing the effect of different *Agrobacterium* strains/binary vectors, *vir* gene induction agent and co-cultivation medium on the transformation frequency. Putatively transformed plants were recovered from co-cultivated embryogenic calluses on selection media containing hygromycin (50 mg/l for barley), geneticin (30 mg/l for wheat) or bialaphos (4 mg/l for wheat and barley). Cellular and molecular analyses indicated that transformation frequencies of 0.3 – 3% were achieved in barley. This demonstrated that commercial cultivars were amenable to *Agrobacterium*-mediated transformation. The new transformation systems will have relevance to corresponding research in other cereals and grasses. This work was supported by a grant from the UK's DEFRA.

P-1378

Highly Efficient Somatic Embryogenesis and Regeneration in Monocots: Application to Genetic Engineering. F. EUDES[1], A. Laroche[1], S. Acharya[1], L. B. Selinger[2], K. J. Cheng[3]. [1]Agriculture and Agri-Food Canada, Lethbridge, AB, T1J 4B1 Canada; [2]University of Lethbridge, Lethbridge, AB, Canada; and [3]Institute of BioAgricultural Sciences, Academia Sinica, Taipei, Taiwan. E-mail: eudesf@em.agr.ca

A novel regeneration protocol using recurrent embryogenesis was developed for cereals. The protocol includes a rapid induction of direct somatic embryogenesis of scutellum cells followed by secondary embryogenesis, germination of primary and/or secondary embryos and regeneration of normally growing green and fertile plants. Tissues are plated on very rich and well defined media to induce and direct development of embryogenic cell and/or cell clusters, and to support growth of these embryogenic cells. The four media used provides a specific sequence of plant growth regulators at different concentrations and ratios based on differential requirement of the successive developmental stages of the barley and wheat embryos. Tissues are transferred to the next medium only when they reach the appropriate developmental stage. This protocol is applicable to all monocots tested so far and is also cultivar independent following testing on winter and spring hexaploid wheat, durum wheat, *Triticum monococum*, wheat amphiploids, barley, oat, sorghum and corn. The regeneration efficiency is 10–12 primary embryos per excised scutellum and up to 10 secondary embryos per primary embryo. An obvious application of a cultivar independent rapid regeneration technique in monocot is toward genetic transformation. This eliminates the difficulties of genotype-dependent regeneration encountered by many research groups around the world. Repetitive cycles of direct embryogenesis effectively substitute for the callus phase found in indirect embryogenic systems, and make repetitive embryogenesis a powerful method by which to obtain a wide range of transgenic plants from transformed sectors within a primary somatic embryo and consequently minimizes the formation of chimeric plants.

P-1379

Cryopreservation of Cocoa (*Theobroma cacao* L.) Somatic Embryos Using Encapsulation-dehydration. J. FANG and A. Wetten. Department of Horticulture & Landscape, School of Plant Sciences, University of Reading, Whiteknights, Reading RG6 6AS, UK. E-mail: ahr00jf@reading.ac.uk

Since maintenance of cocoa (*Theobroma cacao* L.) germplasm in seed form is restricted due to their limited shelf life cryopreserved *in vitro* cultures represent an attractive alternative target for long-term storage. The objective of the present study was to utilise isolated, staminode-derived somatic embryos for cryopreservation rather than floral tissues which may or may not give rise to embryos. Somatic embryos of genotype LCTEEN162/1010 were first pregrown for 16 hours on a medium enriched with 0.3M sucrose, followed by encapsulation within 3% calcium alginate beads including a combination of two cryoprotectants (0.4M sucrose and 2M glycerol) for an hour. Encapsulated embryos were then subjected to 5–7 hours fast desiccation over silica gel and to fast freezing in liquid nitrogen for 30 minutes. Finally, frozen embryos were thawed for 5 minutes at 35°C before being plated on a recovery medium. 30–40% post thaw recovery has been recorded through both primary and secondary embryogenesis pathways. Transmission electron microscopy has revealed the nature of sub-cellular damage that led to the regeneration failure of inappropriately pretreated somatic embryos. These findings indicate that cryopreservation of isolated cocoa somatic embryos represents an effective means of securely maintaining clones of a species for which the efficiency of somatic embryo initiation is currently highly genotype dependent.

P-1380

A Model of the Organ Differentiation and Pure Callus Culture Under In Vitro Conditions Published in the Years 1936 and 1938: O. ORSÓS, the Forgotten Researcher. MIKLÓS G. FÁRI. Centre of Agricultural Sciences, University of Debrecen, 4032 Debrecen, Böszörményi út 138, and Agroinvest Co. Ltd., Budapest, HUNGARY. E-mail: efari@axelero.hu

Sachs' hypothesis (1880) postulated special organ forming substances, furthermore, Haberlandt (1902) supposed that totipotence is an ability of the cells to develop into whole plant. The existence and mechanism of those phenomena has been one of the main topics of plant physiology around the first part of the 20eth century. Fundamental techniques of plant tissue culture were elaborated by White (1934), moreover by Gautheret (1939) and Nobecourt (1939). Results of Ottó Orsós related to organ regeneration and to callus formation were communicated in the periodicals Protoplasma (1936) and Biologisches Zentralblatt (1938), and the present author compared them with the contemporary papers of the former mentioned researchers published between 1932 and 1940. As a disciple of Á. Paál in Budapest, Orsós improved the concepts and methods of Haberlandt and White. He elaborated a multi-functional *in vitro* model of callus-induction as well as of organ-regeneration on cubes of undifferentiated tissue made from kohlrabi tubers. In 1936 he demonstrated by this system that the *"wound-hormone"* of Haberlandt (1922) is identical with tyrosine. Subsequently, Orsós isolated different extracts from the kohlrabi tuber. He used the extracts as regulators inducing either *in vitro* shoot organogenesis or, alternatively, rhizogenesis of kohlrabi cubes (1938). The experiments have been completed by histogenetic proofs too. He stated that *"shoot inducing principles are to be distinguished from root inducing ones."* Orsós produced on a modified substrate of White *"a continuously growing pure callus isolate, which could transferred and maintained"* (1938). Orsós died in 1939, and the wartime are the reasons of oblivion of his name.

P-1381

The Effects of Different Concentrations of Thidiazuron and Sucrose on Shoot Proliferation and Flowering of *Dendrobium nobile* Second Love (Orchidaceae) *In Vitro*. W. M. FERREIRA and G. B. Kerbauy. UNITINS/IB, Department of Botany, University of Sao Paulo, Brazil. E-mail: wmelo100@yahoo.com

Several hybrids of *Dendrobium nobile* are very important for the orchid industry in a number of countries, including Brazil. In the last few decades, various synthetic compounds which influence growth and morphogenesis have been developed. One of these compounds is a substituted diphenyl urea known as "thidiazuron" (TDZ) which is now among the most active cytokinin-like substances for plant tissue culture. The addition of a carbon source into the culture medium is usually necessary when growing plant parts *in vitro* and sucrose has been the most commonly used carbohydrate in plant tissue culture media. Taking that into consideration, the objective of the present study was to evaluate the effects of TDZ and sucrose on shoot proliferation and flowering of *D. nobile* plants *in vitro*. Shoots obtained from micropropagated plants of *D. nobile* Second Love were grown on Vacin and Went (1949) basal medium, modified by substituting $Fe_2(C_4H_4O_6)_3$ for 27.8 mg/L Fe EDTA, and supplemented with the micronutrients of MS media (1962), 0.4 mg/L thiamine and 100 mg/L myo-inositol. TDZ (0 to 3.6 microM) and sucrose (0 to 4%) were added to the culture medium in different combinations. TDZ alone was not capable of inducing flower initiation. However, the interaction between TDZ and sucrose markedly enhanced both flowering and shoot proliferation. TDZ at 1.8 microM and 2% sucrose made up the best combination for shoot proliferation whereas 2% sucrose combined with either 1.8 or 3.6 microM TDZ resulted in the best treatments for flower induction. Sucrose and TDZ concentrations above 2% and 0.45 microM, respectively, did not exert great effects on shoot dry matter accumulation. These results suggest that the method described herein is efficient for large-scale micropropagation of *D. nobile* Second Love and provides interesting material for further studies on the transition from vegetative to reproductive stage of *Dendrobium* species.

P-1382

Use of Seed Germination from Mature Fruits as a Model for the Introduction of *In Vitro* Plant Biology to Middle School Science Students. J. E. Finer, A. D. Taylor, J. E. Stump, and J. J. FINER. OARDC/The Ohio State University, Wooster, OH 44691. Email: finer.1@osu.edu.

With the multiple public misunderstandings of plant biotechnology, an introductory plant biotechnology exercise may be useful to educate middle school students (and possibly their parents) to some of the basic aspects of *in vitro* culture. Clearly, DNA introductions and manipulations are outside of the arena of possibilities for many classroom exercises. However, placement of tissue in culture could be used as a simple, introductory exercise for plant biotechnology. Since the main problem in establishing cultures in a classroom setting is contamination, seeds from mature fruits were selected as the subject for this study. Fruits were purchased from the local grocery store and surface sterilized by washing first with soapy water and then spraying with a 70% alcohol solution. For excision of seeds, fruits were cut open with a kitchen knife, which was first dipped in alcohol. Seeds were removed from the fruit using either an alcohol-dipped forceps or latex gloves, which were first sprayed with 70% alcohol. Seeds were plated on media containing MS salts, B5 vitamins, 3% sucrose and 0.2% Gelrite as a solidifying agent. Some media contained GA to break dormancy of the immature seeds in some fruits. Seed excision experiments were initially conducted in a laminar flow hood and then on an open laboratory bench. Establishment of aseptic cultures and seed germination were highly variable and apparently dependant on the status of the staring materials. Seeds from kiwi, cantaloupe and star fruit were very poor performers, yielding very low germination and/or high degrees of contamination. Seeds from different types of peppers and tomatoes tended to have low contamination but germination varied from fruit to fruit. Seeds from citrus (grapefruit, lemon, lime, orange) gave the most consistent results, with low contamination rates and intermediate germination.

P-1383

Micropropagation of Papaya Selections from Mature Trees in a Backcross-breeding Program. M. FITCH[1], T. Leong[2], N. Saito[2], G.Yamamoto[2], S. Maeda[1], S. Ferreira[3], R. Jacobson[4], and P. Moore[1]. [1]Pacific Basin Agricultural Research Center, ARS, USDA, 99–193 Aiea Hts. Dr., Aiea, HI 96701; [2]Hawaii Agriculture Research Center, 99–193 Aiea Hts. Dr., Aiea, HI 96701; [3]University of Hawaii, 3190 Maile Way, Honolulu, HI 96822; and [4]Brigham Young University, 55–220 Kulanui St., Kahuku, HI 96731. Email: MFITCH@HARC.HSPA.COM, MFitch@pbarc.ars.usda.gov

Papayas have traditionally been multiple-planted as seeds or seedlings and thinned to a single hermaphrodite per hole since sex expression in hybrids is 1 female to 1 hermaphrodite. The practice is wasteful of seed, resources, and the labor required for overplanting and thinning. In addition, hermaphrodite individuals can vary from poor to excellent. For example, tetraploids, sterile, and/or carpellodic Kamiya hybrid trees occur in approximately 1/300 planting holes along with outstanding lines. An attempt to increase yield of high quality fruit is being made by the development of a cropping system based on micropropagation of selected outstanding lines. The procedure is especially important because the backcross population of Kamiya F_1 to BC_3 trees varied greatly in fruit and fruit column quality, and an inbred, uniform seed line has not yet been developed. Selections from among mature hermaphrodite Laie Gold, the name of a Kamiya F_1 line, were made based on visual evaluation of tree and fruit column appearance and on fruit size, flavor, firmness, sweetness, texture, and color. Lateral branches of the selections were rinsed overnight in running water, agitated in commercial bleach solution, and explanted onto medium containing carbenicillin, naphthalene acetic acid, and benzyladenine. The survival and proliferation rates of the shoot explants were determined as were the rooting and ex vitro survival rates. A timescale from root initiation in vitro to field-ready potted plants was established. Limited numbers of micropropagated selections were placed in field tests in a few locations to evaluate broad adaptability. The performance of these selections ranged from good to outstanding. However, before undertaking large-scale micropropagation, the selections should be shown to perform well, at least in some locations. The selected papaya lines will be compared with several unselected lines to determine if any of them show improved performance and yield in various locations in Hawaii.

P-1384

Field Screening of Cold-Hardy Palms for Micropropagation. DAVID A. FRANCKO & Kenneth G. Wilson. Dept. of Botany, Miami University, Oxford, OH 45056. E-mail: franckda@muohio.edu

In 1998 we began at Miami University an evaluation of currently-available cold-hardy palm species/varieties under USDA Zone 6 conditions. One objective is the identification, development, and micropropagation of 'next generation' commercial cultivars with improved cold hardiness. Our research plan incorporates ten replicated on-campus plots (microclimate = Z7a), several off-campus plots in colder microclimatic areas (Z6a), and 15 species/25 varieties of clump and tree palms (N = 140) ranging from second-year juveniles to reproductive-age individuals. Nearly all field-grown palms have survived winter conditions to date (including a lows of −12°F in Z6a sites and −2°F in warmer campus sites in Jan 2000). Clumping palms, including needle palm (*Rhapidophyllum hystrix*) and several varieties of scrub palmetto (*Sabal minor*) were the most cold hardy, with minimal leaf damage down to zero degrees F. *Trachycarpus fortunei* cultivars/varieties defoliate below about 5°F, and generally recover from subzero F temperatures. Cabbage palmetto, Mediterranean fan palm, and other 'Zone 8' palms exhibit high survivorship, and foliage can be overwintered with appropriate winter protection (wind barriers and leaf mulch). By field screening large numbers of plants under extremely rigorous conditions we have identified individuals of each species that exhibit enhanced cold-tolerance phenotypes. Preliminary data suggest that these individuals can be cloned via shoot bud tissue propagation. We acknowledge support from the Ohio Plant Biotechnology Consortium.

P-1385

Ultrasonic Treatment Damages the Surface Layer of Explants and Induces Shoot Regeneration in Squash Cotyledon Explants. V. GABA, X. Xia, S. Singer, I. Fischer, A. Gal-On, and G. Ananthakrishnan. Department of Virology and SEM Laboratory (I. F.), ARO Volcani Center, POB 6, Bet Dagan 50250, Israel. E-mail: vpgaba@volcani.agri.gov.il

Ultrasonic treatment (0.5–2 min) stimulated the shoot regeneration process from cotyledon explants from recalcitrant batches of squash (*Cucurbita pepo* L.) from low levels (15%) to the high levels (70%) observed in non-recalcitrant batches. Regeneration by ultrasound was stimulated *in vitro* for explants from a number of commercial squash cultivars on Murashige and Skoog (1962) medium augmented with benzyladenine. Ultrasonic treatment also stimulated massive growth of the explant as in non-recalcitrant material. Ultrasonic treatments of 5 or 10 minutes also promoted regeneration and growth—now accompanied, however, by massive hyperhydration. The shoots regenerated without ultrasound treatment were very small, and the explants not regenerating shoots often formed a microscopic bud-like structure, which could regenerate a fasciated shoot on transfer to elongation medium. Ultrasonic treatment (2 min) resulted in multiple shoot production from the explant, giving total shoot production several times greater than the control. Observations by scanning electron microscope showed that 2 min ultrasound changed the joint area between epidermal cells, apparently by removing some of the waxy surface from the cotyledon. The 10 min vitrifying ultrasound treatment caused further removal of the waxy deposit, not only from between epidermal cells, but also from the external cell surface as well. Notably, both the 2 min and 10 min treatments did not cause gross surface injury to the explants, with the exception of damaging stomatal complexes. However, a non-physiological treatment of 30 min ultrasound produced significant surface damage: areas of the external cell wall from the outside of the explant were pealed off, and additionally produced isolated exploding cells and cracks in the explant surface. This is the first report of stimulation of *in vitro* regeneration by ultrasound treatment.

P-1386

Genetic Fidelity of *Arabidopsis thaliana* (L.) Heynh. Plants Regenerated *via* Direct Somatic Embryogenesis. M. D. GAJ, L. Kapka, A. Kiwior, and M. Kwasniewski. Department of Genetics, Silesian University, 40–032 Katowice, Poland. E-mail: mmdgaj@us.edu.pl

Somaclonal variation in *A. thaliana* regenerants was evaluated with the use of isozymes and DNA markers: RAPD and AFLPs. The analyzed plants were obtained through a rapid and efficient process of direct somatic embryogenesis (DSE) induced in culture of immature zygotic embryos of Columbia ecotype. Zymograms of three enzymes: esterases, GOT and LAP were evaluated in 56 regenerants (R1) and their progenies (R2 and R3). To analyze genetic polymorphism at DNA level, RAPD and AFLP markers were applied with the use of 36 and 5 primer combinations, respectively. The progeny of 27 and 30 regenerants were tested in RAPD and AFLP analysis, respectively. Isozymes and DNA markers were also monitored in control combination, which included 30–50 seed-derived plants. In total 18 062 of isozymatic loci, 7 762 of RAPD and 2 040 AFLP bands were scored in the regenerants and their sexual progeny. Changes in zymograms of esterases and GOT (including band intensity, lost or appearance of a new band) were detected in R1 and R2 plants but not in R3 progeny indicating their epigenetic character. All of the analyzed DNA amplification products displayed no aberration in banding pattern among the tested regenerants, their progeny and the control plants. The obtained results provide evidence for high genetic fidelity of the *Arabidopsis* plants regenerated *via* DSE and thus recommend this system as a valuable regeneration method in biotechnology of this model plant. This work was supported by research grants of the State Committee for Scientific Research (KBN) No. 6 PO4B 013 18 Poland.

P-1387

Evaluation of Mannose Selection for Sugarcane Transformation. M. GALLO-MEAGHER, K. Chengalrayan, and A. M. Abouzid. Agronomy Department, University of Florida, Gainesville, FL 32611–0300. E-mail: MGMEA@GNV.IFAS.UFL.EDU

Sugarcane transformation has relied heavily upon the use of negative selectable marker genes such as neomycin phosphotransferase II (*npt II*) and *bar* which confer resistance to particular antibiotics and herbicides, respectively. A positive selectable marker gene such as *manA* that codes for phosphomannose isomerase and provides transformed cells with a metabolic advantage over non-transformed cells may allow for higher sugarcane transformation efficiency. Initially, mannose alone and in combination with sucrose was examined for an effect on sugarcane callus growth. Modified MS medium containing 3 g/L mannose plus 20 g/L sucrose, which resulted in 80% callus browning and subsequent callus death, was used for selection in our transformation study. Sugarcane callus was co-bombarded with an untranslatable sugarcane mosaic virus strain E coat protein gene construct and *manA*. Following selection, transgenic calli were regenerated on MS medium containing 2.5 micromolar thidiazuron and 3 g/L mannose. Approximately, 100 transgenic shoots were obtained and plantlets were successfully transferred to the soil following rooting on MS medium containing 5 mg/L indole-3-butyric acid. Molecular characterization is currently underway to determine gene copy number, co-integration rates, and expression levels.

P-1388

Regeneration Through Organogenesis, Somatic Embryogenesis and *Agrobacterium* Mediated Transformation in Cowpea (*Vigna unguiculata*). A. GANAPATHI and R. Prem Anand. Department of Biotechnology, Bharathidasan University, Tiruchirappalli, TN, India – 620 024. E.mail: GANAP@BDU.ERNET.IN, BIOVENGI@REDIFFMAIL.COM

Direct and indirect regeneration from cotyledon explants has been established for Cowpea. Callus cultures initiated from immature cotyledon explants on MS+ B_5 medium containing IBA and Kn each at 2.0mg/l concentration when transferred to medium containing 0.1mg/l Zeatin produced adventitious shoots. The regenerated shoot buds were grown in a medium containing BAP (0.5mg/l) and GA_3 (0.1mg/l) for elongation. The shoots were transferred to IBA (0.5mg/l) fortified medium for root induction. For multiple shoot induction from immature cotyledon, they were dissected into two parts namely proximal and distal portion. Multiple shoot induction was achieved only from the proximal part of the cotyledon, while, the distal part produced compact callus that failed to regenerate. Maximum (60%) multiple shoot induction response was observed when the proximal part was cultured in the MS medium containing 0.1 mg/l of Zeatin and 1.0 mg/l BAP. The explants were transferred to half strength MS liquid medium supplemented with 1.0 mg/l of GA_3 for shoot elongation The same medium also favoured profuse root development. Embryogenic callus was induced from leaf explant when cultured on MS+ 2,4-D at 1.5 mg/l. The greenish white friable embryogenic callus when subcultured on MS liquid medium containing 0.5 mg/l of 2,4-D produced globular, heart and torpedo shaped embryos. Further development and maturation of torpedo shaped embryos occured in 0.1 mg/l 2,4-D, 3% Mannitol and 1.4 mg/l ABA. Germination was achieved in solidified MS half strength medium (0.15% gelrite) with 0.5 mg/l Zeatin, 3% Mannitol, 1.4 mg/l ABA. The regeneration protocols thus developed were used for *Agrobacterium* mediated gene transfer by using the binary vectors containing *nptII*, *Bar* and *GUS* marker genes. Both direct regeneration and Somatic embryogenesis pathways were used to recover transgenic plants. The former is more efficient than the latter. Selection agents such as kanamycin, Hygromycin and PPT were compared. The PPT was found more effective selection agent than the other two. The above study thus illustrated reproducible protocols for transformation and regeneration, thus paving the way for genetic manipulation of cowpea.

P-1389

Use of Mature Embryos as Explants for Regeneration of Barley (*Hordeum Vulgare* L.). SEEDHABADEE GANESHAN[1], Monica Båga[1], Bryan Harvey[2], Brian Rossnagel[3], Graham Scoles[2], and Ravindra Chibbar[1]. [1]National Research Council, Plant Biotechnology Institute, 110 Gymnasium Place, Saskatoon, SK, S7N 0W9; [2]Department of Plant Sciences, 51 Campus Drive, Saskatoon, SK, S7N 5A8; and [3]Crop Development Centre, 51 Campus Drive, Saskatoon, SK, S7N 5A8. E-mail: ravi.chibbar@nrc.ca

The main objective of this study is to develop genetic transformation systems for four commercially important barley genotypes, *viz.*, CDC Dawn and CDC McGwire (feed barley), CDC Copeland and BT459 (malting barley). Suitable *in vitro* culture systems were investigated based on immature scutella, leaf-base tissues and mature embryos as explants. Explants were cultured on different callus induction media for the production of highly embryogenic callus. Subsequently, several other media were used to improve shoot regeneration. For callus induction, a modified MS medium supplemented with 1 mg/L 2,4-D and 30 g/L maltose was found to be optimal for all four genotypes using immature scutella and leaf-bases as explants. Regeneration of shoots from callus derived from both explants was found to be optimal on a modified MS medium supplemented with 0.05 mg/L 2,4-D + 1 mg/L BA or 0.05 mg/L 2,4-D + 2 mg/L BA. The regeneration frequencies were comparable for both types of explants, with shoot production ranging from 1.5–3.5 shoots per explant. With further modifications to the growth regulator levels in the media, the leaf-base and mature embryo explants could be induced to produce multiple shoots directly, without an intervening callus phase. Depending on the genotype, a two- to eight-fold increase in shoot production could be observed. These explants were tested in transformation experiments using particle bombardment to deliver constructs expressing the BAR, NPTII or GUS genes. For transformation by *Agrobacterium tumefaciens*, the strain LBA4404 harbouring the plasmid pTOK233, carrying the GUS, NPTII and HPT genes was used. Transformation of all three types of explants by particle bombardment and/or *Agrobacterium* has been demonstrated by transient GUS expression. Several putative transformants that have survived selection, have been shown to be PCR positive for the genes of interest. Detailed molecular analyses are currently being conducted to confirm stability and expression of the transgenes.

P-1390

Regeneration and *Agrobacterium*-mediated Transformation of *Avena sativa* L. GOKARNA GHARTI-CHHETRI and Olof Olsson. CMB, Gothenburg University, Box 462, SE-405 30 Gothenburg, Sweden. E-mail: gkn.ghch@molbio.gu.se, olof.olsson@molbio.gu.se

Protocols were established for callus cultures of various oat cultivars (Adamo, Belinda, Birgitta, Freja, Matilda, Petra and Stork). An *in vitro* system was based on 2,4-D containing callus induction medium, 3- and 6-day old leaf/mesocotyll joints (LB/M) and growth in the dark. Shoot regeneration was thereafter initiated in a medium with a defined auxin/cytokinin combination. Roots were regenerated in a hormone free medium. Complete regeneration was obtained from Belinda, Birgitta, Freja, Matilda and Stork. Several strains and co-cultivation conditions were tested to genetically transform oat with *Agrobacterium tumefaciens*. More specifically, LB/M were co-cultivated with an *Agrobacterium* strain carrying binary 4x35::uidA and 4x35:: m-gfp5-ER vector constructs. A general GUS activity, or green fluorescence, was observed in calli derived from these explants followed by, after a few weeks, a more localized expression, still present several months later. Thus, from these experiments it is clear that *Agrobacterium* mediated gene transfer and integration into the oat genome indeed does occur. However, the hygromycin selection used was toxic, leading to watery, non-regenerable calli. To avoid this selection system, a new selection procedure for oat callus based on the *E. coli manA* gene was developed. A binary vector carrying a 4x35::*manA* gene cassette was constructed and transformed into oat by *Agrobacterium*. Very recently, regenerating shoots growing on 30mM mannose were obtained from callus derived from this co-cultivation, but not from non-transformed control callus. PCR analysis shows the integration of the *manA* gene.

P-1391

The Development of New Potato Cultivars through the Regeneration of Periclinal Chimeras. MARGY J. GILPIN & Anthony J. Conner. New Zealand Institute for Crop and Food Research Ltd, Private Bag 4704, Christchurch, New Zealand. E-mail: gilpinm@crop.cri.nz

A chimeric plant is a type of genetic mosaic, which has a mixture of cells from two or more different genotypes. Important natural chimeras are known in clonal crops such as potatoes and apples and many arose spontaneously as "sports". Our research investigated whether such chimeras could be deliberately synthesised using potatoes as a model system. In vitro grafting and subsequent mixed callus culture between two cultivars, Iwa (which produces tubers with white skin and flesh) and Urenika (which produces tubers with purple skin and flesh), has resulted in the production of potatoes which have purple skin and white flesh. Preliminary investigations using RAPD analyses indicate the putative chimeric plants have DNA markers unique to both parental cultivars. This technology has the potential to create novel potato clones with improved agronomic, processing and consumer appeal. Cultivars of potatoes with high quality tuber flesh could be surrounded by skin from another genotype or species which has improved appearance or disease resistance.

P-1392

Biochemical Description of Pea Somatic Embryogenesis. M. GRIGA, J. Horacek, and H. Klenoticova. Plant Biotechnology Department, Agritec Ltd., Zemedelska 16, CZ-787 01 Sumperk, Czechia. E-mail: griga@agritec.cz

Physiological quality of mature somatic embryos affects strongly germination and subsequent optimum plant development, and thus the efficiency of somatic embryogenesis-derived technologies. Among others, the proper physiological state is guaranteed by the accumulation of storage substances typical for sexual seed and it is influenced by previous *in vitro* culture (induction, development and maturation phase). In this paper we characterize the process of pea somatic embryogenesis using basic simple protocol (regeneration from shoot apical meristems) with the further aim to improve the physiological quality of embryos by manipulation of critical culture factors. Initial explants, callus tissue and individual developmental stages of somatic embryos (characterized by their distinct morphology, size and fresh weight) of five dry-seed pea and five canning pea cvs. with good embryogenic competence were analyzed for accumulation of starch and its components amylose and amylopectin, and for biosynthesis of storage proteins. In addition, developmental expression of thirteen enzymes was recorded (AAP, AAT, ACP, ADH, AMY, DIA, EST, LAP, MDH, PGM, PRX, SDH, SOD). The accumulation of starch and seed storage proteins increased during somatic embryo development despite of the fact that no special maturation culture treatment was applied. Major storage proteins typical for pea seed were detected (convicilin, vicilin, legumin alpha, lectin beta). Four enzymes exhibited uniform spectra in analysed samples. All other enzymes exhibited differential expression during somatic embryo development. In general, cotyledonary somatic embryos exhibited highest activities and usually richest spectra of particular enzymes analyzed. A decrease of starch and protein content sometimes recorded in cotyledonary stage may be attributed to mobilisation of storage reserves in the beginning of precocious germination of somatic embryos, which process still may not be apparent morphologically. Potential culture manipulations for improving physiological maturity of pea somatic embryos are disscussed.

P-1393

Improving the Efficiency of Early Stages of Somatic Embryogenesis of *Coffea arabica*. EMMA C. GUZMÁN RAMOS and Elliott H. Birnbaum. Albert Katz International School for Desert Studies and Department of Dryland Biotechnologies, Jacob Blaustein Institute for Desert Research. Ben-Gurion University of the Negev, Sede Boker, 84990, Israel.

Mass clonal propagation is often the key to economically viable utilization of valuable plant germplasm. Priority has, therefore, been given to developing low cost cloning technologies for many important crops. One method that has proven to be highly efficient is somatic embryogenesis. The van Boxtel and Berthouly (1996) protocol for somatic embryogenesis of *Coffea arabica* L. begins with a two step "embryogenic callus induction" stage (ECI). The stage involves culture of leaf discs for one month on a callus induction medium, followed by culture for two months on an embryogenic callus induction medium. Our work indicates that by modifying the media formulations and environmental conditions for ECI, the process of production of embryogenic callus becomes more efficient. a. Casein hydrolysate and malt extract, components of the media for both steps, appear to be unnecessary. b. PPFD of 30 or 72 $\mu mol/m^2/s$ during the callus induction step resulted in greener and more nodular calli and in greater quantity than that produced in the dark. per the original protocol. c. Incorporation of $CaCl_2$ (9 mM) in the embryogenic callus induction medium accelerated the appearance of friable embryogenic calli. These calli proliferated rapidly and in high density following transfer to liquid culture, in the callus proliferation stage. This research was supported by the Bona Terra Foundation.

P-1394

Efficient Plant Regeneration through Morphogenesis in Japanese Commercial Variety of Wheat. TAKASHI HAGIO, Shin'ichiro Kamachi, Tomohiro Yamada, Takahiro Asano, and Yutaka Tabei. National Institute of Agrobiological Resources, Kannondai Tsukuba, Ibaraki 305–8602 Japan. E-mail: hagio94@nias.affrc.go.jp

Twenty-one Japanese commercial varieties of wheat were examined for their response in tissue culture, and the tissue culture system was optimized. The cultures were initiated from immature embryos taken approximately two weeks after flowering. Regeneration occurred at higher frequencies when cytokinins (kinetin or 6-benzyladenine) had been added in the callus induction medium(modified MS medium), followed by regeneration medium devoid of growth regulators. The addition of maltose, proline, glutamine and asparagine also enhanced shoot formation, but the addition of cytokinins to regeneration media did not improve shoot formation. In many cases, shoot formed by the formation of adventitious buds and distinct somatic embryogenesis was not observed. Five varieties (Norin No. 35, Norin No. 40, Norin No.67, Norin No.81, Norin No. 83) showed high frequency of callus formation and plant regeneration. Self-fertilized seeds of "Norin No.67" were obtained and advanced to the next generation (R1). Tissue culture response of the R1 plants were examined. Plant regeneration frequency of the R1 (82%) generation was significantly higher than that of the R0 (53%). Foreign genes (35S + nptII) were introduced into immature embryos of "Norin No.67, R1" using particle gun (Biolistic PDS-1000/He). At present, 12 geneticin-resistant plants were regenerated and four plants showed positive response in PCR analysis. Application of this knowledge should facilitate the generation of transgenic wheat.

P-1395

Propagation of *Corynanthera flava* via Somatic Embryogenesis. KACUNG HARIYONO[1], Beng Tan[1], and Siegy Krauss[2]. [1]Department of Environmental Biology, Curtin University of Technology, Perth, Western Australia, 6845, Australia, and [2]Kings Park Botanic Garden, Perth, West Australia, Australia. Email: kacunghariy@hotmail.com

Corynanthera flava ("Golden Cascade") is a small, willowy myrtle, endemic to the sandplains, some 200 km north of Perth, Western Australia. Flower stems of this wildflower are premium as cut flowers in Japan; these are harvested exclusively from the sparsely distributed wild populations. Cultivation can be realized if successful propagation can be demonstrated. Several conventional and *in vitro* propagation techniques are being explored, in particular, the efficacy of somatic embryogenesis. Three-week-old seedlings were divided into three explant types (cotyledon, hypocotyl, or root) and cultured on MS medium containing 1.0 mg/L 2,4 D. Somatic embryogenesis in induced calluses was observed after 6 weeks. Somatic embryo formation was highest in calluses from cotyledon explants, followed by the calluses from hypocotyl sections. Calluses from root explants did not produce any somatic embryo. Efficacy in somatic embryogenesis differed between seed parents and between populations; significantly, within a seed parent, the number of somatic embryos arising from callus was similar. Germination was successful on basal MS medium containing 2% sucrose. Zero survival of plantlets during acclimatization was ascribed to their leaves lacking cuticle and functional stomates.

P-1396

Selection for Pepper Shoot Regeneration by Mannose. Joo Yean Kim, Min Jung, Byung Whan Min, Seung Gyun Yang, CHEE HARK HARN. Biotechnology Center, Nong Woo Bio Co., Ltd., Yeoju-kun, Kyonggi - do, Korea E-mail: chharn@nongwoobio.co.kr

Several selection strategies have been developed to avoid the use of antibiotics and herbicide. One of them is to use mannose as a carbon source in the media. Many plant species can not metabolize mannose to other sugars because phospho-mannose isomerase (PMI) that converts mannose to fructose is not present in plants. PMI gene from E. coli has been cloned and, recently, a few plants such as maize and sugar beet have been successfully transformed with PMI as a selection marker. This approach would lead the social issue of GMO production to less concern. To apply this technique to pepper plants that have been known as one of the most difficult crops to be transformed with the present selection pressure, the regeneration efficiency has been examined with combined concentrations of sucrose and mannose in the media. Fifteen to 20g/L of mannose mixed with 20 g/L of sucrose in either shooting and elongation media would be the best condition for properly selecting the regeneration of pepper shoots. The regeneration rate, however, was variable depending upon the pepper inbred line. More data will be discussed on the poster.

P-1397

High-Frequency Plant Regeneration from a Long-Term Root Culture (Super Roots) of *Lotus corniculatus*. M. HASHIGUCHI[1], T. Kawano[1], Ryo Akashi[1], Shyun-Shyun Hoffmann-Tsay[2], and Franz Hoffmann[2]. [1]Faculty of Agriculture, Miyazaki University, Miyazaki 889–2192, Japan and [2]Department of Developmental and Cell Biology, University of California, Irvine, CA 92697–2300. E-mail: rakashi@cc.miyazaki-u.ac.jp

Super roots of *Lotus corniculatus* are a fast growing legume root culture that allows continuous root cloning, direct somatic embryogenesis and mass regeneration of plants under entirely growth regulator-free culture conditions (Akashi et al., 1998). These features are unique to root cultures and are now stable expressed since the culture has been isolated almost 5 years ago. Super roots switch from exclusive root proliferation to shoot production upon transfer to light and stationary condition. Lateral root formation continues at a reduced rate while embryos and shoots are forming. When treated with 0.5 mg/L 6-benzylaminopurine (BAP), lateral root formation, somatic embryogenesis and *in vitro*-nodulation are strongly inhibited. Instead, shoots form at an increased rate matching the spacing pattern typical for lateral roots. Shoots, not embryos, are also formed on stem and leaf explants of super roots regenerants. The response to hormones, the positioning of structures and the mode of emergence suggest that root-derived somatic embryos as well as root nodules are developmentally closely related to lateral roots, i.e., are produced by retuned initiation sites. Akashi et al. (1998) Theor. Appl. Genet. 96:758–764.

P-1398

Abstract has been withdrawn

P-1399

Various Nutrient Media and Color Illumination Effect on *In Vitro* Shoot Production in Water Chestnut (*Trapa* sp.). A. HOQUE and S. Arima. Faculty of Agriculture, Saga University, Saga-840–8502, Japan. Email: kd0102@edu.cc.saga-u.ac.jp

Shoot production efficiency through cotyledonary node as well as nodal explants were studied under various nutrient media and color illumination condition in water chestnut (*Trapa japonica* Flerov). Under the various nutrient media (MS, MMS, MMS1, MSMA, N6, B-5 and White's; all are Sigma product) conditions, MSMA supplemented with 2.7 μM BA, 0.5 μM NAA, and 0.5 μM GA3 showed the best performance for shoot proliferation than other media supplemented with above growth regulators combination. MSMA produced the maximum 21.6 ± 0.8 and 14.6 ± 0.6 shoots, and other media produced the average 7.8 ± 0.3 and 2.2 ± 0.1 shoots from the cotyledonary node as well as nodal explants respectively after 6 weeks of culture. The competence of cotyledonary node as well as nodal explants for the induction of multiple shoots under the influence of various nutrient media as well as plant growth regulators have been observed. Under the various color (white, blue, mixed, red and infra-red) illumination, mixed color illumination showed the early and higher percentage of shoot proliferation. Fresh and dry weight were also significantly higher under mixed color condition. Media were also contained MSMA with 2.7 μM BA, 0.5 μM NAA, and 0.5 μM GA3 in all color illumination. Cultures were maintained at 28±1 C under a 16-h photoperiod of 60 μmol m-2 s-1 provided by white fluorescent tubes for nutrient media and various color provided by color illumination system (MIL C1000T, Sanyo EC, Japan) respectively. Shoots produced *in vitro* rooted 100% of the time in a liquid MS medium with 5.4 μM IBA and the plantlets were established successfully in the field.

P-1400

Use of Elite Genotypes and HiII-elite Hybrids in *Agrobacterium*-based Transformation of Maize. MICHAEL E. HORN, Robin Harkey, Amanda Vinas, Carol Drees, Kathy Beifuss, Donna Barker, and Jeffrey Lane. ProdiGene, 101 Gateway Blvd., Suite 100, College Station, TX 77845. E-mail: mhorn@prodigene.com

Hybrid embryos resulting from crosses between a highly regenerable maize germplasm (HiII) and certain elite inbreds were treated with Agrobacterium tumefaciens containing the gus and pat genes under the control of two different constitutive promoters, respectively. The elite inbred lines consisted of six Lancaster and three Stiff Stalk types. Hybrid embryos from all three Stiff Stalk lines gave transgenic events at various frequencies; two not significantly lower than with HiII embryos. Only one Lancaster type out of six attempted showed successful transformation as part of a hybrid with HiII and the frequency was quite low. The resultant transgenic events showed many characteristics of the elite inbred parent including more aggressive rooting, thicker stems, and taller stature than plants derived from HiII events. The hybrid T0 plants also exhibited excellent tassel development in the greenhouse with abundant pollen shed. Seed set in the greenhouse was significantly (4–5 fold) higher than with HiII transformants. Attempts to transform embryos derived from self or sibling crosses of the four inbred lines, successful as hybrids with HiII, did not produce any transgenic events. Nevertheless, T0 plants having ~50% elite genomic contribution perform nearly as well in the greenhouse as seed-derived elite parents and offer a significantly reduced time line for transprotein product development. We had predicted only a modest increase in seed set using hybrid embryos, but we were surprised by the magnitude of the improvements throughout the entire system.

P-1401

Investigation of Direct Somatic Embryogenesis in Genera *Medicago*. A. V. IANTCHEVA, A. L. Barbulova, M. G. Vlahova, H. Trinh, S. Brown, and A. I. Atanassov. AgroBioInstitute, 2232 Kostinbrod, Bulgaria; Institut des Sciences du Végétales, CNRS, UPR 2355 bat. 23/24, avenue de la terrasse, 91198 Gif-sur-Yvette Cedex, France. E-mail: anelia_iantcheva@agrobioinstitut.org

Somatic embryogenesis provides an ideal experimental process for investigation of plant differentiation as well as the mechanisms of expression of totypotency in plant cell. All the process strongly depends of the culture media and the conditions, genotype and primary explant. Tetraploid *Medicago* species may be regenerated in vitro via direct somatic embryogenesis but they possess high degree of heterozygosity and large genome size. On the other hand tetraploid alfalfa has also been developed as an experimental model for the studies of somatic embryogenesis. Autogamous annual medics are closely related to alfalfa, but they are diploid, selfpollinating and possess short life cycles. Hence, they are more suitable for molecular, cellular and genetic investigations as long as clones with high morphogenic capacity are available. Procedures for direct production of somatic embryos from diploid and tetraploid *Medicago* species have been developed. It involves wounding of the plant tissue and 2,4D treatment of different explants in liquid media for both *Medicago* species and also was found promotive role of TDZ for direct embryo formation on solid media for diploid medics. The procedure in liquid media effect of wounding and following 2,4D treatment are the trigger to promote competent cells in the explant to divide rapidly and to form globular embryos. Other factor influence to proportion of competent cells within a tissue most notably is genotype. The genome size and polysomaty of the starting explant tissue assessed by flow cytometry was found to influenced on the embryogenic competence and stability of regenerated plants in diploid *Medicago* species. Single cell suspension cultures of transgenic *Medicago falcata* plants carrying the *gus A* gene under the control of two promoters *cdc2aAt* and *cyc3aAt* from cell cycle regulating genes, have been obtained in order to visualize cells active and competent for cell division and to confirm the asymmetry of the first cell division in embryogenic competent cell. The process of embryo formation was characterized from the single cell until conversion to plantlets. The fraction of highly embryogenic single cell in suspension culture of transgenic *Medicago truncatula* plants expressing *gfp* reporter gene were obtained after transformation of leaves. Using this single cell suspension culture early events of direct somatic embryo formation were studied. The fluorescent protein largely facilitates observation of these living cells and localization of the nuclei in first asymmetric embryogenic division.

P-1402

Transformation of Sugarbeet Using Cell Suspension Cultures. S. D. IVIC[1*], J. W. Saunders[2], and A. C. Smigocki [1]. [1]USDA, ARS, Molecular Plant Pathology Laboratory, Beltsville, MD 20705, and [2]USDA, ARS, Dept. of Crop and Soil Sciences, Michigan State University, East Lansing, MI 48824. *Graduate student- University of Belgrade, Yugoslavia. E-mail: sivic@asrr.arsusda.gov

A sugarbeet transformation method was developed using particle bombardment of short-term suspension cultures. Callus obtained from leaf discs of greenhouse-grown plants was propagated in liquid medium for 2 weeks and bombarded 1 day after sieving and plating onto agar medium. After 2 to 5 days callus was passed to medium containing either kanamycin or paramomycin and maintained on the selection media for different lengths of time. The introduced DNA contained both *uidA* gene (GUS) under the control of either the osmotin (OSM) or proteinase inhibitor II (Pin2) gene promoter and *npt II* gene under the control of the 35S promoter. Transient GUS expression monitored 2 days after bombardment showed 900 to 3000 blue units per bombarded plate of 0.2 g of suspension cells. GUS expression decreased significantly during the next 7–14 days of culture. Stably transformed calli were obtained as early as 3 weeks following bombardment at a frequency of 0.25–9 calli per bombarded plate. Presence of the introduced genes was confirmed by PCR analysis. To induce regeneration of plants, calli are being maintained on media with varying plant growth regulator composition.

P-1403

Somatic Embryogenesis of *Iris* spp.: Anatomy Study. SLADJANA B. JEVREMOVIC and Ljiljana Radojevic. Institute for Biological Research, Department of Plant Physiology, 29 Novembra 142, 11 060 Belgrade, Yugoslavia. E-mail: SLADJA@IBISS.BG.AC.YU

One of the major problems of monocot somatic embryogenesis is that during the regeneration process, two different regeneration pathways seemed to be observed: somatic embryogenesis and / or organogenesis. The results of this study should brighten this problem. Induction of somatic embryogenesis of *Iris halophila*, *I. pumila* and *I. sibirica* were achieved in leaf-base culture of *in vitro* grown plants on solid MS medium (Murashige and Skoog, 1962) supplemented with 2,4-D and kinetin (1.0 mgL^{-1}, each). Transversal leaf-base section, 30 days after induction, showed formation of nodules in the region of leaf mesophylle. Cross section of each nodule showed several layers of vacuolated, round cells at the surface. Under these layers, region of procembryogenic meristem cells were observed. The centre of nodule was with vacuaolated cells. Formed nodules were transferred to MS medium without hormones for further differentiation. Somatic embryo and shoot—like structures were observed. Detailed anatomical studies have shown that shoot-like structures were precociously germinated somatic embryos. Serial section of shoot like-structures reveal bipolarity, but with rudiment developed radicula. These findings thus confirm that morphogenesis of irises, at first, proceeded in the direction of somatic embryogenesis and later as and organogenesis.

P-1404

In Vitro Morphogenesis of Mature Nodal Explants of *Sesbania sesban*. AJAY K. JHA[1], K. Nanda[2], and S. C. Gupta[3]. [1]Department of Horticulture & Landscape Architecture, Colorado State University, Fort Collins, CO 80523–1173; [2]Dept. of Botany, Daulat Ram College, University of Delhi, India; and [3]Dept. of Botany, University of Delhi, Dehli, 110011, India. Email: ajk1965@hotmail.com

Micropropagation technique has revolutionized agriculture and forestry with its unending significance from evolving disease-free better varieties to elite clones which can be preserved and propagated in minimum span of time and space in a cost-effective method. Experiments were conducted in 10-year-old woody species *Sesbania sesban*, nodal explants. The explants were surface sterilized (0.1% $HgCl_2$ for 10 min. followed by 5 min. treatment with 70% alcohol) and inoculated on different basal media (B_5, MS, and Nitsch), of which Nitsch medium was most responsive in terms of shoot production and average number of shoots and its length per explant. At different BA treatments, 0.5 mg/l proved optimum for shoot formation and highest number of shoots per nodal explant *via* callus phase. Excised callus when subcultured on the same medium, shows shoot bud formation. However, Kn (1.5 mg/l) supplemented medium developed direct shoot differentiation with 12.5 ± 7.9 average number of shoots per explant, and the morphology of the shoots were normal. On the basis of this observation, Kn (1.5 mg/l) was selected as optimum for direct shoot differentiation. 1–4 cm long shoots harvested from 90-day-old cultures of nodal segments were transferred to different combinations of IBA in Nitsch medium for rhizogenesis. The best response was achieved on 2 mg/l IAB, formed 50% rooting with very little callus and better root growth, plantlets of this origin were successfully transferred to the field, survived initially four months in culture room and for another three months in the field.

P-1405

Micropropagation of *Scutellaria integrifolia* L., a Medicinal Skullcap. NIRMAL JOSHEE and Anand K. Yadav. Agricultural Research Station, Fort Valley State University, 1005 State University Drive, Fort Valley, GA 31030–4313. Email: josheen@mail.fvsu.edu

Since the 1980s, the herbal industry experienced an exponential growth. About 80% of the world population relies on phytomedicines for primary health care. *Scutellaria* species have been used in the traditional medical systems of China, Korea, India, Japan, several European countries, and North America. *Scutellaria baicalensis* is the most widely used species while others (*amoena, hypericifolia, likiangensis, rehderiana* etc), have also been utilized beneficially. Flavonoids from *S. baicalensis* roots showed inhibitory effect on HIV-I, HTLV-I, and mouse skin tumor promotion. Anti-inflammatory activity of *Scutellaria* products has been associated with its capability to specifically inhibit COX-2 (cyclooxygenase-2) enzyme. About 20 *Scutellaria* species grow in Georgia and the bordering states, but little is known about their medicinal values. Some of these species are even endangered, threatened or rare. We are developing *in vitro* systems for local *Scutellaria* species to study clonal multiplication and secondary metabolite biosynthesis. Shoot apices bearing immature fruits were collected. Leaf and nodal sections were used to initiate cultures in dark with $2mgL^{-1}$ BA in the MS medium. Explants were sterilized with 10% Clorox for 20 min and were further soaked in 1% PPM for 30 min. Cultures were incubated for one week in dark and for additional two weeks in light at 23–25C. In this study, only few nodal explants responded, forcing out axillary buds that were allowed to elongate and then were used as explants. Explants swell and became rough on the surface at the end of three-week incubation. At this point, explants were transferred to MS+2 mgL^{-1} BA + 0.1 mgL^{-1} NAA for two weeks under light. Explants developed little callus with many green shoot buds that elongated into shoots upon transferring to basal MS medium for 3–4 weeks. The 3–5 cm long microshoots were excised and transferred to two rooting media (MS+ 2 mgL^{-1} IBA, 1/2MS+ 2 mg L^{-1} IBA) and incubated during first week in dark and then in light. Two weeks later, all shoots developed roots. *In vitro* raised plants are being established in the greenhouse.

P-1406

In Vitro Shoot Regeneration From Seedling Shoot Meristem Explants of Broccoli (*Brassica oleracea* var. *Italica*) Cultivars. S. M. KELKAR, W. N. Jane, and L. F. O. Chen. Institute of Botany, Academia Sinica, Taipei, Taiwan, 115. E-mail: kelkarswati@hotmail.com

Inter-cultivar variation in morphogenic response is an important barrier in biotechnology mediated crop improvement of Broccoli. In order to overcome this problem we used seedling meristem (SM) explants. Use of seedling meristems ensured shoot formation in 80–100% explants irrespective of cultivar used. Effect of 6-Benzyl Adenine (BA) concentration, age of explants and inhibition of ethylene synthesis by silver nitrate ($AgNO_3$) on shoot regeneration in seedling meristem (SM) explants of two cultivars of Broccoli was studied. BA at all concentrations tested resulted in multiple shoot formation by axillary sprouting or adventitious shoot induction. The origin of shoots was confirmed by scanning electron microscopy. BA at 5.0 mgl^{-1} and 10 day old seedlings yielded best results for regeneration. Addition of $AgNO_3$ enhanced the percentage of adventitious shoot induction in both cultivars.

P-1408

Conservation of the Sandy Point Orchid (*Psychilis macconnellia*) through Tissue Culture. J. A. KOWALSKI and T. W. Zimmerman. University of the Virgin Islands-Agricultural Experiment Station, St. Croix, USVI 00851. E-mail: jkowals@uvi.edu

The Sandy Point Orchid (*Psychilis macconnellia*) is an endangered plant species that is indigenous to St. Croix, United States Virgin Islands. The population of this orchid is in great decline due to a number of factors including land development, private collectors and natural disasters such as hurricanes and fires. A seed tissue culture system was developed for this orchid which can be replicated by a home or nursery owner. Seeds were removed from the mature green seed capsule and placed into 1/2 MS media with vitamins, 2% sucrose, 0.1% peptone, 1% activated charcoal and 4g/L agar. The seeds germinated and began producing protocorms after two months in culture. First true leaves and roots appeared after five months. The plants were grown in culture for a total of twelve months. The orchids were then hardened off, placed in a commercial orchid media mix and transferred to a greenhouse. Preliminary results indicate that the orchids can be released back into their natural environment within eighteen months.

P-1409

Promotion of Photosynthesis, Growth, and Development of *Coffea arabusta* Somatic Embryos by Photoautotrophic (Sugar-free) and High CO2 Concentration Conditions. Ayako Uno[1], CHIERI KUBOTA[2], and Toyoki Kozai[1]. [1]Department of Bioproduction Science, Chiba University, Matsudo, Chiba 271–8510, Japan. [2]Department of Plant Sciences, The University of Arizona, Tucson, AZ 85721–0036. E-mail: chierik@aol.com

Somatic embryogenesis is a key technology for mass production of woody plants. However, its wider application is limited mainly due to low percent conversion to plantlets and their subsequent slow growth rate. Somatic embryos with chlorophyllous cotyledons could be cultured photoautotrophically (Afreen et al., 2001). Our research intends to promote the growth and development of *Coffea arabusta* somatic embryos by controlling the in vitro environment under photoautotrophic conditions. Photosynthetic ability of the cotyledonary embryos was confirmed by measuring net photosynthetic rates (NPR) at 100 micro-mol m^{-2} s^{-1} PPF. The NPR were well described with a mathematical model indicating that the NPR became saturated at 3000 to 5000 micro-mol mol^{-1} CO_2. According to these observations, cotyledonary embryos were cultured photoautotrophically for 61 days under a CO_2 concentration of 400, 1500, or 5000 micro-mol mol^{-1} at 100 micro-mol m^{-2} s^{-1} PPF using a liquid medium with a porous substrate. Cotyledonary embryos successfully grew photoautotrophically at all CO_2 levels. Percent conversion to plantlets was enhanced and reached 55 to 60% at 1500 or 5000 micro-mol mol^{-1} CO_2 under photoautotrophic conditions while it was 17% under conventional conditions (20 g/L sucrose in an agar-gelled medium, 400 micro-mol mol^{-1} CO_2 and 30 micro-mol m^{-2} s^{-1} PPF). The fresh and dry weights, averaged per culture (embryo or plantlet), increased with increasing CO_2 concentration. The dry weight per culture under photoautotrophic conditions at 5000 micro-mol mol^{-1} CO_2 was almost 6 times of that cultured under conventional conditions. An 80% survival rate was exhibited when transplanted to ex vitro (70% R.H., 30°C air temperature and 300 micro-mol m^{-2} s^{-1} PPF) subsequent to the photoautotrophic culture at 5000 micro-mol mol^{-1} CO_2 without acclimatization. Photoautotrophic and high CO_2 concentration conditions were shown to promote photosynthesis, conversion of cotyledonary embryos to plantlets, growth, and ex vitro survival of *Coffea arabusta*.

P-1410

Agrobacterium-mediated Transformation of Super Roots of *Lotus corniculatus*. Y. Kutsuna[1], M. Hashiguchi[1], RYO AKASHI[1], S.-S. Hoffmann-Tsay[2], and Franz Hoffmann[2]. [1]Faculty of Agriculture, Miyazaki University, Miyazaki 889–2192, Japan and [2]Department of Developmental and Cell Biology, University of California, Irvine, CA 92697–2300. E-mail: rakashi@cc.miyazaki-u.ac.jp

Super roots of *Lotus corniculatus* are a fast growing legume root culture that allows continuous root cloning, direct somatic embryogenesis and mass regeneration of plants under entirely growth regulator-free culture conditions (Akashi et al., 1998). These features are unique to root cultures and are now stable expressed since the culture has been isolated almost 5 years ago. In several years of experimentation, all our attempts to directly transform super roots with *Agrobacterium* failed for unknown reasons. Only recently, by using super root-derived callus as acceptor tissue, we were able to demonstrate transient expression of a reporter gene (GUS) in a tissue derived from super roots. GUS expression was also visible in roots and shoots regenerated from transformed calli. The super root character is stable maintained through the callus phase. Roots derived from these calli can serve as explants for new super root cultures. This stability of the callus phase makes it unlikely that transformation was made possible through the loss of protective genetic information, such as a spontaneous *A. rhizogenes* infection, through callus initiation. Akashi et al. (1998) Theor. Appl. Genet. 96:758–764.

P-1411

A Thin Cell Layer Culture System for the Rapid and High Frequency Direct Regeneration of Sugarcane and Other Monocot Species. PRAKASH LAKSHMANAN*, R. Jason Geijskes*, Adrian R. Elliot**, Lifang Wang*, Michael G. McKeon*, Rhylee S. Swain***, Zara Borg*, Nils Berding***, Chris P. L. Grof**, and Grant R. Smith*. *David North Plant Research Centre, Bureau of Sugar Experiment Stations, 50 Meiers Road, Indooroopilly 4068, Australia; **CSIRO-Plant Industry, Long Pocket Laboratories, Indooroopilly 4068, Australia; and ***Meringa Sugar Experiment Station, BSES, Bruce Highway, Gordonvale 4865, Australia. E-mail: PRAKASHL@BSES.ORG.AU

Sugarcane (*Saccahrum* spp. interspecific hybrids), a graminaceous crop, accounts for nearly 70% of sugar production worldwide. The potential of tissue culture to enhance conventional sugarcane breeding and molecular crop improvement has received considerable recent attention, but its use has been limited by the high incidence of somaclonal variation in plants regenerated using the current callus-based tissue culture systems. To minimise somaclonal variation we have developed a rapid, genotype-independent and high frequency direct shoot regeneration system in sugarcane using a thin cell layer culture approach. In this system, 1–2 mm transverse sections of young leaves or inflorescence were cultured on MS medium enriched with various concentrations of a-naphthaleneacetic acid (5–20 μM), chlorophenoxyacetic acid (5–10 μM) and 6-benzyladenine (1–5 μM), singly or in combinations. Plantlets regenerate directly from the explant in 8–10 weeks. Developmental polarity and morphogenic competence of explant were the key determinants regulating shoot regeneration. Up to 2000 plantlets can be produced from a single sugarcane leaf roll. Plants have been successfully regenerated from 20 sugarcane genotypes and two genotypes each of wheat and sorghum. Field trials indicate no significant variation in stool weight and sugar yield between tissue culture-derived and traditionally propagated plants. This novel culture system has also proved to be a rapid method for virus elimination, and is the underlying technology for the most efficient sugarcane transformation system described to date.

P-1412

Vegetative Phase Change in Lily Bulblets Regenerated In Vitro. M. M. LANGENS-GERRITS, G. J. De Klerk, and A. F. Croes. Applied Plant Research, Flower bulbs, PO Box 85, 2160 AB Lisse, The Netherlands. E-mail: M.M.Langens@ppo.dlo.nl

In the ontogenetic development of lily, three different phases can be distinguished: the juvenile phase, the vegetative adult phase and the generative adult phase. We have studied the transition from the juvenile to the vegetative adult phase in lily bulblets regenerated in vitro. Juvenile bulblets sprout with only one or a few leaves, whereas vegetative adult bulblets sprout with a stem with several leaves. The apex of adult bulblets is characterized by the presence of a stem primordium with elongated internodes and a tunica-corpus structure in the apical meristem. Juvenile bulblets first developed competence to respond to the inductive signal. Temperature was a major factor influencing this process. Competence developed during a long period (more than 6 weeks) at high temperature (25°C). Induction and expression of the phase change was also strongly influenced by the temperature and occurred during a short period (2 weeks) at low temperature (15 °C). Small bulblets (under 100 mg fresh weight) never formed a stem whereas large bulblets (over 800–900 mg) always formed a stem under inducing conditions. In the intermediate weight class, large bulblets more often formed a stem than small ones but phase change was also influenced by other factors: (1) a high sucrose concentration, (2) a large explant, (3) a prolonged period for competence development stimulated phase change, (4) a low concentration of MS-minerals allowed phase change to occur in small bulblets. Tissue culture systems allow careful examination of plant developmental processes as all culture conditions can be strictly controlled and interaction with the rest of the plant is restricted to the explant. Lily provides an excellent model to study vegetative phase change because the factors influencing vegetative phase change can be clearly distinguished from those inducing flowering. Another avantage of tissue culture is the relatively short time required for bulblet regeneration.

P-1413

Changes in DNA Methylation During Somatic Embryogenesis in *Cucurbita pepo* L. D. LELJAK-LEVANIC, N. Bauer, and S. Jelaska. Department of Molecular Biology, Faculty of Science, University of Zagreb, Zagreb, Croatia 10000. E-mail: dunja@zg.biol.pmf.hr

In many plant species somatic embryogenesis is initiated in the presence of a high concentration of auxin (2,4-D). Removal or decreasing the concentration of auxin from the culture medium is an important factor in the development of the advanced stages of somatic embryos. A correlation between exogenous auxin and DNA hypermethylation, as well as embryo stage specific changes in DNA methylation, has been demonstrated. The goal of this study was to find out whether high methylation in the early stages of embryo development is a consequence of auxin treatment, or whether it is a characteristic feature of those stages. Three embryogenic lines of pumpkin (*Cucurbita pepo* L.) were established: a) an auxin independent line (PEDC)—induced and maintained on hormone-free MS medium with only NH_4Cl as a source of nitrogen; b) a line (DEC)—induced and maintained on MSC medium supplemented with 2,4-D; and c) a habituated line (HEC) – derived from line DEC and maintained on hormone-free medium. The progression of embryogenesis in three lines was controlled by auxin presence/absence and by modification of reduced/unreduced nitrogen. Changes in cytidine methylation in embryogenic lines of pumpkin cultivated on different media were analyzed by random amplification of genomic DNA after digestion with enzymes *Hpa* II or *Msp* I. The highest methylation was detected in all lines on the media that enabled proembryo and globular stages: 2,4-D-free MS supplemented with NH_4Cl, and MSC with 2,4-D. The level of DNA methylation decreased during embryo maturation, which occurred on MSC media without 2,4-D but with added conventional nitrogen sources. Methylation decreased considerably on the medium supplemented with 5-azacytidine; however, the embryogenic potency was preserved. The plant regeneration obtained on MSC medium with IAA corresponded to a slight increase of DNA methylation. The results showed that two independent control factors, reduced nitrogen (NH_4Cl) and 2,4-D, supported a high level of DNA methylation in early embryo stages.

P-1414

New Approach for Biolistic: Can Functionalized Particles Improve Cell Transformation? SOPHIE LE ROUX. Aventis CropScience, 14/20 rue Pierre Baizet BP 9163, 69263 Lyon Cedex 09, France. E-mail: sophie.le-roux@aventis.com

Two main technologies are available for gene introduction in plants: *Agrobacterium tumefaciens* and the biolistic approach. With the second method, the standard protocol (1) extensively uses accelerated metal particles coated in a mixture consisting of DNA, calcium and polyamines. We have evaluated new kinds of particles called "functionalized particles" (2) in order to study their impact on transformation efficiency and DNA pattern integration. Particles functionalized with aminosiloxanes allow us to form a direct complex with DNA avoiding the use of polyamines and calcium. Different kinds and sizes of functionalized particles were evaluated in transient and stable expression on soybean tissues. Studies have shown that using less DNA, compared to the classic protocol, results in both transient and stable expression being increased by up to 4 times. The pattern of DNA integration was also evaluated by southern blot. The interest and the potential of these particles in plant transformation are discussed in this poster. (1) Klein T.M., Fromm M., Weissinger A., Tomes D., Schaff S., Sletten M. & Sanford J.C. *Proc. Natl. Acad. Sci.* 1988, 85, 4305–4309. (2) US Patent number 6,068,980: Nitrogen-containing silicone useful for compacting nucleic acid sequences and use for transforming cells.

P-1415

Regenerability of Wild *Nicotiana* Species. BAOCHUN LI and Troy Bass. Tobacco and Health Research Institute, University of Kentucky, Cooper and University Drives, Lexington, KY 40546–0236. E-mail: bli2@uky.edu

The objective of this study was to examine the regenerability of wild *Nicotiana* species. Leaves from in-vitro-grown seedlings were cut into pieces (approximately 3 mm by 3 mm) and cultured on shoot regeneration medium (MS salts plus B5 vitamins, 2.5 mg L^{-1} BAP and 1.0 mg L^{-1} IAA) under light for three weeks, and then subcultured onto the same medium for another three weeks. Percentage of leaf pieces producing shoots and average number of shoots produced per generating leaf piece were determined. The regenerability of as many as 69 wild *Nicotiana* species is being examined. Initial results on 13 of the 69 *Nicotiana* species indicated that five species (*N. benthamiana*, *N. glauca*, *N. glutinosa*, *N. sylvestris* and *N. tomentosiformis*) had 50–100% regeneration, five species (*N. alata*, *N. clevelandii*, *N. excelsior*, *N. langsdorfii* and *N. occidentalis*) had 1–49% regeneration, and three species (*N. arentsii*, *N. knightiana* and *N. rustica*) had no regeneration. With regard to the number of shoots produced per generating leaf piece, three species (*N. glauca*, *N. sylvestris* and *N. tomentosiformis*) had more than 10, two species (*N. benthamiana* and *N. glutinosa*) had 5–9, and five species (*N. alata*, *N. clevelandii*, *N. excelsior*, *N. langsdorfii*, and *N. occidentalis*) had 1–4. These results suggest that wild *Nicotiana* species differ significantly in their regenerability.

P-1416

Agrobacterium tumefaciens-Mediated Transformation of Corn (*Zea mays* L.) Multi-shoot Cultures. WENBIN LI, Pat Masilamany, K. J. Kasha, K. Peter Pauls. Biotechnology Division, Department of Plant Agriculture, University of Guelph, Guelph, ON, Canada N1G 2W1. E-mail: wenbinli@uoguelph.ca

Agrobacterium-mediated transformation has been very successful with a few monocotyledonous species but is not very efficient with corn. An *Agrobacterium*-based transformation protocol had been described using corn immature embryos as the starting material (Ishida, 1996 and Negrotto, 2000). Our interest is to develop an alternative procedure using multi-shoot cultures derived from young corn seedlings (Li et al., 2002). A potential advantage of using multi-shoot callus is that the regeneration response of shoot-tip cultures is relatively genotype-independent, thus, greatly enhancing the applicability of the transformation system. Bacterial strains AGL1, EHA101, C58C1rif and LBA4404 harboring plasmids pIG121-Hm or pBU-35S.IG were used with cultures initiated from 11 Ontario inbreds that had high multi-shoot formation efficiency. Twelve factors that affect transformation were evaluated. Agropine-type *Agrobacteria* were the most competent to infect multi-shoot tissues (8 fold stronger than octopine-type strain). The corn inbreds displayed significant differences in their responses to *Agrobacterium* infection. The stage of the multi-shoots, a culture supplement of proline and the addition of Selwet-70 during co-culture greatly stimulated transformation frequencies. A strong correlation between shoot-tip susceptibility to *Agrobacterium* and the proportion of cells in G1 phase was also observed. Moreover, Southern hybridization revealed that corn transformants contained a single copy or two copies of insert DNA. The frequency of transformants based on regenerated plants was around 1%.

P-1417

Efficient Transformation of Somatic Embryos of Rose via *Agrobacterium tumefaciens*. XIANGQIAN LI, Sergei F. Krasnyanski, and Schuyler S. Korban. Department of Natural Resources & Environmental Sciences, University of Illinois, 310 ERML, 1201 W. Gregory, Urbana, IL 61801. E-mail: Xli7@uiuc.edu

The effects of various antibiotics (kanamycin, carbenicillin, and cefotaxime) on regeneration from different tissues of rose (*Rosa hybrida* cv. Carefree Beauty), including leaf, undifferentiated callus, and primary somatic embryogenic callus, were first investigated to determine optimal antibiotics and concentrations. Then, the cloning vector pCAMBIA 2301 containing the *uidA* gene coding for B-glucuronidase (GUS) gene expression driven by the CaMV 35S promotor and the *npt II* gene for kanamycin resistance was immobilized into *Agrobacerium tumefaciens* strain GV3101, and used for transformation of leaf, undifferentiated callus, and primary embryogenic callus. Following cocultivation and selection of these various explants on a regeneration medium containing 100 mg/L kanamycin and 500 mg/L cefotaxime, secondary somatic embryogenic explants were induced. Upon transfer of secondary somatic embryos to a kanamycin-free regeneration medium, putative transgenic plants were induced. A total of 120 putative transgenic plants were developed from 30 primary embryogenic callus within a 12 month period. These were subjected to histochemical analysis, and 10 plants exhibiting strong GUS activity were identified. Stable integration of the transgene was confirmed by both PCR and Southern hybridization.

P-1418

A High Endogenous Glucose Content May Play an Important Role in Rice Shoot Regeneration Induced by Osmotic Stress. LI-FEI LIU and Wen-Lii Huang. Department of Agronomy, National Taiwan University, Taipei, 106, Taiwan. Department of Biotechnology, Fooyin Institute of Technology, Kaohsiung Hsien 831, Taiwan. E-mail: lfliu@ccms.ntu.edu.tw

In previous studies, we discovered that the shoot regeneration ability of rice callus could be dramatically promoted by osmotic stress treatment. This provides an alternative concept that the growth and differentiation of cells could be modulated by the cellular physiological water status. We are thus interested in what cellular physiological events occurred during this process. At first, the sucrose and starch metabolisms in rice (*Oryza sativa* L. cv. Tainan 5, TN5) callus were studied because carbohydrates are the main energy source in plant cells. The results showed that fresh weight, water content, cellular water and osmotic potentials of callus all decreased significantly in highly regenerable callus which was induced on MS basal medium supplemented with 10 μM 2,4-D and 0.6 M mannitol (TN5-M_6). Besides, the starch and soluble sugar contents in TN5-M_6 callus were higher than those in un-regenerable callus, induced on the same medium without mannitol. Then, a sudden increase of glucose content was found in TN5-M_6 the first day after the callus was transferred to regeneration medium. Simultaneously, the activities of sucrolytic enzymes, sucrose synthase and acid invertase, were higher and they may have responded to the increase of glucose content. It is suggested that the sudden increase of glucose content may play an important role in rice shoot regeneration.

P-1419

Regeneration of *Sabal palmetto* from Meristem Explants through Organogenesis. MEEPA A. LOKUGE, David A. Francko, and Kenneth G. Wilson. Department of Botany, Miami University, Oxford, OH 45056. E-mail: lokugem1@muohio.edu

Sabal palmetto (Family Areaceae), the state tree of Florida and South Carolina is widely-cultivated in the US throughout USDA Zone 8. With relatively minor improvements in cold tolerance and cold acclimation, this palm is a potential candidate for general horticultural use in colder areas. The only method of propagation of *Sabal palmetto* is through seeds and there is a great deal of variation among seedlings. For the production of identical clones in large numbers the tissue culture technique offers great potential. An attempt has been made to develop a method for clonal propagation of *Sabal palmetto* using meristem tissues. A meristem from a three-year-old plant was dissected under aseptic conditions and allowed to grow in callus induction medium (modified Murashige and Skoog medium) for about four weeks. The resulting calli were transferred into media with gradually decreasing concentrations of 2,4-dichloropheonoxy acetic acid (2,4-D) in four week intervals. Incubation was carried out in dark at 29°C. Cytokinin – benzylaminopurine (BAP) was introduced with the lowest concentration of 2,4-D used. Calli did not form somatic embryos with gradually decreasing concentrations of auxin but started to form number of shoots when introduced into the medium with low concentration of BAP. Therefore, this shoot regeneration is possibly through organogenesis. Introduction of other cytokinins including thidiazuron (TDZ) did not show similar observations. Upon initiation, shoots were transferred into shoot regeneration medium. Shoots are growing successfully in the shoot germination medium supplemented with low concentration of BAP under 16 h photoperiod. Root formation in all the shoots has occurrenaturally. Therefore, introduction of root induction medium was not required for these plantlets. Although the protocol needs to be refined for large scale production of clonal plants this is the first report for plant regeneration of *Sabal palmetto*. We are investigating the possibility of using various explants with different media compositions for the consistent clonal propagation of *Sabal palmetto*.

P-1420

Assessment of Somaclonal Variation in Somatic Embryo-derived Cocoa (*Theobroma cacao*). C. RODRIGUEZ-LOPEZ, A. Wetten, and M. Wilkinson. Dept of Agricultural Botany, University of Reading, RG6 6AS, UK. Email: aar99cmr@rdg.ac.uk

Now that efficient somatic embryogenesis of cocoa (*Theobroma cacao*) has been established this method of clonal propagation is set to underpin the future multiplication and distribution of novel elite clones of this crop. Because the somatic embryogenesis procedure is dependent on a protracted callus phase it is the objective of this work to establish the frequency of any somaclonal variation arising in somatic embryo-derived cocoa plants. Duplicate DNA extractions have been made from over 500 somatic embryo-derived cocoa plantlets and these are currently being screened for polymorphisms using a range of molecular procedures: (1) chromosome loss has been monitored using microsatellites belonging to each of the 10 cocoa linkage groups; (2) 24 Inter Simple Sequence Repeat (ISSR) primers have been screened for their appropriateness for the detection of major insertion/deletions and (3) the occurence of single point mutations in four Sequenced Characterized Amplified Regions (SCAR) markers has been assessed using Single Strand Conformation Analysis (SSCA) while an algorithm-based analysis has been developed for the detection of mutant-derived restriction sites. Preliminary analysis of the ISSR band profiles generated with five primer pairs has revealed that less than 2% of the clones screened exhibited somaclonal variation.

P-1421

Genotypic Variation of Maize During Organogenesis In Vitro. C. LÓPEZ-PERALTA, L. Iracheta-Donjuan, V. A. González-Hernández, and E. Cárdenas-Soriano. Laboratorio de Biotecnología Agrícola, IREGEP-Colegio de Postgraduados, km. 36.5 Carr. México-Texcoco, Montecillo, Edo. de México, México. CP 56230. E-mail: cristy@colpos.mx

The *in vitro* organogenic ability (including caulogenesis and rhizogenesis) of eight maize genotypes (5 inbred lines, 2 hybrids and a synthetic variety) was evaluated in this study. The histological origin of shoots was determined also. In all cases, explants were 1 mm thick disks obtained from the shoot apex of seedlings grown *in vitro*. Treatments included three physical states of the culture medium (solid, liquid and liquid with a filter paper support) and several hormones as: GA_3 (0, 1.44, 2,89 and 4.83 μM); three auxins at three levels: NAA (16.45, 26.85, and 37.59 μM), IAA (17.12, 28.53, and 39.95 μM); and IBA (14.76, 24.60, and 34.44 μM). The histological analysis showed that during induction the resulting shoots had been originated from axillary buds; while during the multiplication phase, shoots were formed either from axillary buds or from cortical cells of lateral shoots. The highest capacity for shoot induction was attained by two lines, L6 and L13, and by the hybrid L58xL69, in the liquid medium with filter paper support, but the largest organogenic rate was reached by lines L6 and L16, GA_3 (0.0–4.33 μM) did not affect the rate of shoot multiplication. The best hormonal treatment for root induction was NAA at 26.85 μM in all maize genotypes. This methodology allows to produce *in vitro* 512 maize shoots in 60 days.

P-1422

Using the Maize LEC1 Gene to Recover Transformants in the Absence of Chemical Selection. K. Lowe, G. Hoerster, X. Sun, S. Ellis, *S. Abbit, *K. Glassman, and W. J. Gordon-Kamm. Transformation Research, *Feed and Food Research, Pioneer Hi-Bred, Intl. Inc., Johnston, IA 50131.

Ectopic expression of the maize LEC1 gene leads to increased transformation frequencies, increased callus growth rates and increased re-transformation frequencies. Typically transformed cells are initially compromised and require antibiotic or herbicide selection to enhance their recovery by killing the rapidly growing non-transformed cells. The growth advantage provided by LEC1 overcomes some of these problems and can be used with a screenable visual marker such as a fluorescent protein to recover transformed cells on non-selective medium. By carefully controlling the expression of the anthocyanin pathway using well characterized maize genes it is possible to provide a easily visualized screenable marker that can be used for identifying transformants. Recently, we have used a combination of the maize LEC1 gene and a fusion between the maize R and C1 (CRC) genes to recover transformants at frequencies comparable to that of bialaphos selection. Controlled expression of LEC1 and CRC using either tissue-specific or inducible expression provides a non-herbicide method for selecting transformants. This positive growth transformation system also allows one to recover transgenic plants wherein all the transforming DNA components are derived from maize. Transformation strategies using LEC1 and CRC for event recovery will be discussed.

P-1423

Addressing the Issues of Endogenous Contamination During the Establishment of a Micropropagation System for Sea Thrift (*Armeria maritima*). Leigh Brewin, PAUL T. LYNCH, Aradhana Mehra, and Margaret Farago*. Centre for Environmental and Applied Science Research (CEASR), School of Environmental and Applied Science, University of Derby, Kedleston Road, Derby, DE22 1GB, UK and *Imperial College of Science, Technology and Medicine, Prince Consort Road, London SW7 2BP, UK. E-mail: L.E.Brewin@derby.ac.uk

As part of ongoing investigations into the mechanisms of copper tolerance in *Armeria maritima* (Mill.) Willd and towards the development of hyperaccumulating *A. maritima* lines, *in vitro* culture protocols have been developed. The optimum medium for regeneration of shoots from leaf explants was Murashige and Skoog (MS) medium containing 30g/l sucrose, 0.5mg/l Plant Preservative Mixture(tm) (PPM(tm)), 8g/l agar, supplemented with 0.1 mg/l naphtaleneacetic acid (NAA) and 0.1 mg/l 6-benzylaminopurine (BAP). *A. maritima* has an endogenous microbial contaminant, which has complicated establishment of *in vitro* cultures. The contaminating bacteria were shown to be gram positive and identified as actinomycetes. Addition of PPM(tm) to the culture medium has been shown to be essential for the maintenance of *in vitro* cultures of *A. maritima*, although studies indicate that the action of PPM(tm) is bacteriostatic rather than bacterio-cydal. The concentration of MS salts in culture medium without growth factors significantly influenced the rooting of *A. maritima*. All of the regenerants transferred from *in vitro* conditions to the glasshouse survived and have flowered normally. Histological studies were undertaken to determine the origins of the regenerated shoots. Regeneration appears to be via organagenesis from callus. This may be of significance in future development of *in vitro* derived hyperaccumulating lines.

P-1424

The Cryopreservation and Post-thaw Recovery of Garlic (*Allium sativum*). Graham S. Souch, PAUL T. LYNCH, and Keith Harding*. Division of Biological Sciences, University of Derby, Kedleston Road, Derby DE22 1GB, UK and *Plant Conservation Group, School of Science and Engineering, University of Abertay Dundee, Bell Street, Dundee, DD1 1HG, Scotland. E-mail P.T.Lynch@derby.ac.uk

Cryopreservation offers an important alternative to conventional *in vivo* and *in vitro* approaches to the cryopreservation of garlic germplasm. The use of garlic stem-disc sections (1–1.5 mm thick) provides an alternate source of explants, to the traditional shoot apices, which are capable of producing multiple shoots thus improving the efficiency of garlic cryopreservation. An encapsulation/dehydration approach has been developed for the cryopreservation of garlic stem-disc. Healthy shoot growth was observed with in 3–4 weeks of thawing. This protocol has been used to successfully cryopreserve one commercial and three breeding lines of garlic. Histological examination of stem-disc explants has shown that the shoot regeneration occurs directly from pre-established meristematic areas within the explant without a callus phase. Shoots recovered after cryopreservation have been transferred to compost resulting in plants with morphology comparable to non-frozen germplasm. These plants are currently being grown up to allow the assessment of post-thaw genetic stability.

P-1425

The Effect of Cryopreservation Preculture Treatment on the Oxidative Stress in Plant Cell Suspension Cultures and Post-thaw Regrowth. Zoë A. Hewitt* and PAUL T. LYNCH. Division of Biological Sciences, University of Derby, Kedleston Road, Derby, DE22 1GB, UK and *Present address: Department of Gene Expression & Development, The Roslin Institute, Roslin, Edinburgh EH25 9PS, UK. E-mail: P.T.Lynch@derby.ac.uk

The successful cryopreservation of *Helianthus tuberosus* and *Taraxacum officinale* is dependent upon preculture treatment. For suspension cultures of both species preculture in 0.5 M mannitol resulted in a significant reduction in prefreeze growth and no post-thaw growth, but preculture in 0.5M sucrose resulted in less prefreeze inhibition of cell growth and reproducible post-thaw cell regrowth. The aim of this study was to determine the effects of preculture on the oxidative stress of the suspension cultures. Superoxide dismutase activity was significantly reduced after mannitol preculture, while catalase and peroxidase activities increased significantly. Despite this increase in antioxidant levels in cells pretreated with mannitol the levels of malondialdehyde and Schiff's Bases were higher than in cells pretreated with sucrose. Conversely the level of free proline was significantly increased as a result of sucrose preculture. Similar trends were observed in cultures of both species. The significance of these observations to post-thaw cell regrowth will be discussed.

P-1426

Development of Protocols for the Cryopreservation of Olive (*Olea europaea* L.) Germplasm. Ayesha Siddika, PAUL T. LYNCH, Aradhana Mehra, Carla Benelli*, and Maurizio Lambardi*. Division of Biological Sciences, University of Derby, Kedleston Road, Derby DE22 1GB, UK and *Istituto sulla Propagazione delle Specie Legnose, Consiglio Nazionale delle Ricerche, via Ponte di Formicola 76, 50018 Scandicci (Firenze), Italy. E-mail: P.T.Lynch@derby.ac.uk

Field collections are the traditional *ex situ* germplasm preservation method for woody species of high economic value, such as olive (*Olea europaea* L.). This approach has significant short-comings, including high maintenance costs and the vulnerability of germplasm to damage due to pests and diseases. Therefore the potential for the conservation of olive germplasm using cryopreservation approaches is being explored. Initial experiments using embryogenic tissue (cv Canino) have exhibited post-thaw regrowth and production of somatic embryos after the use of controlled rate freezing or vitrification procedures. Although these protocols are being explored, there are relative few varieties of olive from which somatic embryos/embryogenic callus can be produced, thus there is a need to cryopreserve shoot tips. There has been limited success reported for the cryopreservation of olive shoot tips, a vitrification protocol for olive shoot tips from in vitro grown plantlets (cv Frantoio) resulted in 15% post-thaw survival, but the shoots did not undergo sustained growth. Therefore approaches are being assessed; using a cold tolerant variety, Brisighella, based on combined encapsulation/dehydration and vitrification protocols which have been reported as successful for other woody species.

P-1427

Morphological Study on Somatic Embryogenesis of *Lithospermum erythrorhizon* by Scanning Electron Microscopy. TOTIK SRI MARIANI[1], Kazufumi Yazaki[2], and Hiroshi Miyake[3]. [1]Lab. Physiology, Department of Biology, Institut Teknologi Bandung, Jalan Ganesha 10 Bandung 40132, Indonesia; [2]Lab. Cellular & Molecular Biology of Totipotency, Graduate School of Biostudies, Kyoto University, Kitashirakawa, Sakyo, Kyoto 606-8502, Japan; and [3]Lab. Plant Resources and Environment, Graduate School of Bioagricultural Sciences, Nagoya University, Chikusa, Nagoya 464-8601, Japan. E-mail: totik@bi.itb.ac.id and tsm1966@bdg.centrin.net.id

Lithospermum erythrorhizon is a representative source of shikonin that have antibacterial, anti-inflammatory and anti-cancer activities. The shikonin has been used as an important medicine source in Japan and Indonesia. Plant regenerated from somatic embryogenesis may develop from a single cell so that reduces somaclonal variation. This advantage makes plant propagation more efficient. There has been no report on successful somatic embryogenesis in *L. erythrorhizon*. Somatic embryogenesis in *L. erythrorhizon* was studied by scanning electron microscopy (SEM) to reveal the process and cellular development during the initiation, development and germination of somatic embryo. Initiation of somatic embryogenesis showed the development of embryogenic cells, proembryo, proliferating embryogenic cells (PEC), globular, and heart somatic embryos occurred after 4 weeks of culture in embryo induction medium. The proembryo developed from a single embryogenic cell. Scanning electron micrograph showed the proembryo was composed of small round cells. The PEC was composed of small round cells in the center, surrounded by slightly elongated cells at the periphery. Globular and heart somatic embryos were composed of small round cells. Extracellular materials were observed on the surface of globular somatic embryo. Development of somatic embryos up to torpedo stage occurred after 3 weeks of culture in embryo development medium. Early torpedo stage was composed of small round cells in pre-shoot apic and slightly elongated cells in pre-root apic. This differences showing a polarization of somatic embryo. Germination of somatic embryo occurred after 2 weeks of culture in embryo germination media. Germinated somatic embryo consisted of root, embryo axis and shoot apic. To our knowledge, the scanning electron micrographs of proembryo, PEC, early torpedo and germinated somatic embryo in the present study are the first invention in somatic embryogenesis. We concluded that the above SEM observations could reveal the process and cellular development during somatic embryogenesis. The process underwent sequence as follow: embryogenic cell, proembryo, PEC, globular, heart, torpedo and germinated somatic embryo. The cellular development was the development of small round cells and slightly elongated cells, the formation of extracellular material and the differentiation of those cells leading to the germination of somatic embryo. By understanding the process and cellular development, somatic embryogenesis offers high potential for plant propagation. We are currently continuing this study by performing maturation and conversion of somatic embryo to mass propagate the *L. erythrorhizon*.

P-1428

Embryogenesis from Anther Culture in *Carica papaya*. M. L. MARTINEZ-CARDENAS, A. Martinez, and A. Carmona. Division de Ciencias Biologicas y de la Salud, Universidad Autonoma Metropolitana, Iztapalapa, Mexico, D.F., Mexico. E-mail: martinez.malourdes@correoweb.com

Under stress conditions it have been possible to obtain embryos from anther culture of many species. That conditions induce pollen embryogenesis by androgenesisway. *Carica papaya* has been largely studied to improve it, because of the economical importance, by that, we pretend to obtain androgenesis from anther culture.Anthers, with no mature pollen, were cultures on MS medium with 3,10 or 20% sucrose, with or without 0.1 mg/l ANA or 2,4-D, 0.1 BAP; termic shock and one month darkness condition. It was established five stages for the anther evolution. Callus was formed with 3% sucrose and hormones. On 2,4-D/BAP plus 3% sucrose was observed 14 embryos from 3 anthers. In this study the gametophytic development was deflected to the sporophytic way and callus formation. It was found that the stress condition with 3% sucrose and 0.1mg/l ANA or 2,4-D with 0.1 mg/l BAP yielded androgenesis in papaya.

P-1429

Effects of Hormones on Axillary Buds of Pitaya (*Stenocereus griseus*). M. L. MARTINEZ-CARDENAS, A. Martinez, and A. Carmona. Division de Ciencias Biologicas y de la Salud, Universidad Autonoma Metropolitana, Iztapalapa, D.F., Mexico. E-mail: martinez.malourdes@correoweb.com

Pitaya is a fruit cacti has begun to take attention by its economic importance. In the Mixteca zone of Oaxaca, cacti are principally seen. *S.griseus* can be a sustainable resourse. Many cacti has been propagated by tissue culture techniques. This work was pretendes to induce the axillary buds to plant production. One and three month old shoot from seeds were put on MS solid medium with 1mg/l ANA or AIA, 1mg/l BAP. The vegetative shoots were placed on MS medium with or without 10 mg/l ANA. One month old shoots produced 17.3% vegetative shoots and 28% produced callus on MS medium with ANA/BAP. Three month old shoots produced 53.3% vegetative shoots on MS with ANA or AIA/BAP. 60% vegetative shoots produced roots on MS salts at 4 weeks. We propound that, for *in vitro* propagation is better 1 mg/l ANA or AIA with 1mg/l BAP and exiced the vegetative shoots and place on MS medium.

P-1430

In Vitro Plant Production of *Escontria chiotilla* (jiotilla). M. L. MARTINEZ-CARDENAS, M. C. Cabrera, and A. Carmona. Division de Ciencias Biologicas y de la Salud, Universidad Autonoma Metropolitana, Iztapalapa, D.F., Mexico.

In Mexico there are almost 50% of arid zones, like in Mixteca of Oaxaca. In Mixteca zone there is a fruit cacti with economic trait, that is *Escontria chiotilla* (jiotilla). It's a wild plant and its fruit is collected as sustainable resourse. But it is necessary to propagate for reforestation of areas without vegetation. In this work was pretended to induce plant production from shoots, hypocotiles and cotyledons of jiotilla. Two and three old seedling were excised and shoots, hypocotiles and cotyledons were placed on MS solid medium with 30g/l sucrose, 1mg/l ANA or AIA, 1mg/l BAP and cultured at 16 h light and 27–30°C. vegetative shoots was excised and placed on the same medium. Shoots with 2 month old, produced 80% vegetative shoots on 1mg/l ANA/BAP. Three month old shoots produced 80% of vegetative shoots on AIA/BAP were each shoot produced 8 vegetative shoots. And 60% were produced with ANA/BAP. Callus also was produced on ANA/BAP was better than AIA/BAP. Vegetative shoots produced root at 100% when were placed on ANA/BAP. Hypocotils and cotyledons produced callus at different rate. Three month old shoot on AIA/BAP is better in vegetative shoot production and ANA/BAP is recomended for root production.

P-1430A

A Bar Code Supported Labeling System for Culture Vessels Increases the Safety and Performance of In Vitro Laboratories. SONJA MERKLE(1), Andreas Schlegel(2), Frank Sperrle(2). (1)Reinhold Hummel Tissue Culture Laboratory, Koestlinstr. 121 D-70499 Stuttgart, Germany and (2)CSS—Computer Service Schlegel, Motorstr. 4, D-70499 Stuttgart, Germany. E-mail: (1) merkle@hummel-invitro.de (2) info@css-stuttgart.de

The introduced system was developed by both, micropropagation and data processing specialists for scientific and commercial applications. It has been tested and developed further in practice since September 2001 at the *in vitro* propagation of over three millions of plants. The system meets the following requirements: It rules out any mixing up between clone, varieties or species. Scanning of the labels, followed by immediate processing and analyzing, replaces written records of experimental data. The immediate recording of stock levels and production figures allows a contemporary supervision of relevant criteria like propagation factors, contamination rates or hourly output. Scientific or economic evaluations can be installed, called or changed without knowledge of the underlying data base. Further expansion possibilities: Connection to the own data base system for factoring and accounting. Control of the access to the laboratory or installation of a timekeeping control.

P-1431

Temporary Immersion Bioreactor (TIB-technology): An Efficient Plant Mass Propagation System. M. Escalona, J. C. Lorenzo, M. Daquinta, J. González-Olmedo, R. Rodríguez, C. G. Borroto, D. Castro*, Y. Desjardins**, P. C. Debergh***. Bioplant Center, University of Ciego de Avila, CP 69450, Cuba, *Universidad Católica de Oriente, Colombia, **Centre de Recherche en Horticulture, Quebec, Canada, and ***Department of Plant Production, University of Gent, Belgium. E-mail: mescalona@bioca.unica.cu

Tissue culture technology has become one of the most important way to produce selected varieties of many food crops, ornamental and forestry plants. Currently, plant micropropagation constitutes one of the most effective techniques of commercial application. However, it is confronted with many problems, among them, high production cost, resulted from high handling, low multiplication rate, as well as the high percentage of plants lost or damage in the acclimatisation stage. Thus, cost-effectiveness becomes a challenging issue for plant micropropagators. Temporary immersion technique has been shown to reduce problems usually encountered in liquid culture and produce plantlets with an anatomy comparable to control plants grown outside. Based on this concept, a collective of researcher belong to Bioplant Center adapted a semi-automated system for large-scale propagation of plants. In comparison with conventional micropropagation, they reported a higher proliferation rate, improved labor efficiency, a reduced cost and improved survival rate. Due to the regular pumping of air into the system, Temporary Immersion Bioreactor allows gaseous exchange with the external environment, and this could be responsible for the increased intrinsic plant quality. In order to implement the TIB-technology to commercial production laboratories, it was necessary to optimize the nutritional, ecophysiological and genetic aspects. TIB-technology permits achieve a lot of quantity of plant at low cost.

P-1432

Biotechnology of Small Grain Cereals Using *Agrobacterium*-mediated Transformation. A. NADOLSKA-ORCZYK, A. Przetakiewicz, and W. Orczyk. Plant Breeding and Acclimatization Institute, Radzikow, 05–870 Blonie, POLAND. E-mail: A.ORCZYK@IHAR.EDU.PL

Agrobacterium-mediated transformation system was proved to be effective for dicotyledonous as well as in recent years for monocotyledonous species. The main advantages of the system are: efficiency of transformation and natural mechanism of integration allowing in most cases on precise, frequently one copy T-DNA integration. There are several crucial factors that affect transformation system: plant genotype and regeneration method, bacterial strain and components of transformation vector as well as selection system. Their influence on transformation efficiency and expression of transgenes in three cereal species were compared. Three bacterial transformation systems (strain and plasmid) were tested. Two of them were based on hypervirulent *Agrobacterium* strains (AGL1 and EHA101) and one on commonly used LBA4404 combined with super-binary plasmid pTOK233 (Komari 1990). Transformation susceptibility was established using two cultivars of wheat (Kontesa, Torka), two cultivars of barley (Scarlett, Lot) and one of triticale (Wanad). Regenerating embryoids and plants were selected on media containing appropriate factor: hygromycin or/and kanamycin as well as phosphinothricine. The rate of plant transformation ranged from 0 up to 30 percent and was strongly dependent on cereal species and transformation factors. About 400 adopted in soil and setting seeds plants were obtained. Most of them were triticale plants regenerated on kanamycin selection medium after LBA4404 (pTOK233) transformation. The highest numbers of transgenic wheat plants were obtained after transformation with Agl1 and kanamycin selection as well. Only several plants of barley cv. Scarlett were regenerated, independently of selection factor and *Agrobacterium* system used. Inoculation of about 1000 immature embryos of barley cv. Lot with three bacterial systems factor failed to produce putative transformants. Transgenic character of plants was checked by histochemical analysis of GUS, PCR of *nptII, hpt, bar* and/or *gus* transgenes and confirmed by Southern hybridization. Expression of *gus* (histochemical analysis) and *bar* (Basta leaf painting) in the first and second generation of transgenic plants is currently tested. Komari T. 1990. Plant Cell Rep., 9:303–306.

P-1433

Transient Expression of the *uid* A Gene in Immature Zygotic Embryos and Regeneration of African Varieties of Grain Sorghum (*Sorghum bicolor* L. Moench).
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Grain sorghum (*Sorghum bicolor* L. Moench) is an important indigenous crop uniquely adapted to the semi arid parts of sub Saharan Africa and India. Genetic modification of cereals including sorghum has been achieved. As it is desirable to modify this crop, routinely, for nutritional quality enhancement and disease resistance, improved *in vitro* regeneration and reproducible genetic transformation systems are being investigated for five African varieties. A regeneration protocol was established for immature zygotic embryos (IZEs), which included 100 ml/l coconut water, 1 g/l asparagine, and 2 g/l L-proline and 2 mg/l 2,4 – D in the initiation medium for the production of white compact Type 1 cultures. Somatic embryos formed within 18–21 days after culture initiation, and 10 mg/l silver nitrate induced somatic embryo germination. Stringent selection of embryogenic tissue and frequent transfer reduced the culture period from 28 to 15 weeks for plantlet production suitable for hardening-off, after which they grew into normal fertile plants within 16 weeks. All five varieties were regenerable, and three were selected for transformation. This was undertaken by bombarding IZEs with the pAHC25 plasmid containing the *bar* and the *uid* A genes. Transient expression of the *uid* A gene was improved by a combination of bombarding IZEs 4 to 6 days post excision, 48 h osmotic treatment, a bombardment pressure above 900 kPa, and a DNA concentration of 0.50 microgram DNA per shot. At these conditions, transient expression was approximately 75.4 blue foci per IZE. Selection of transformed cells was undertaken 24 hrs post bombardment on bialaphos-containing induction medium, with a stepwise increase from 0.5 to 2.5 mg /l over a fortnight. Ongoing work is focused on establishing a routine transformation system for sorghum and to improve screening procedures for putative transformants using a combination of the chlorophenol red assay and PCR.

P-1434

Cellular Differences Between Embryogenic Vs Non-Embryogenic Cultures of Winter Oilseed Rape (*Brassica napus* L. spp. *oleifera*).
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Winter oilseed rape (*Brassica napus* L. spp. oleifera) embryoids derived from anther culture undergo secondary embryogenesis in the absence of growth regulators (Loh & Ingram, 1982). Cultures diplodized by colchicine treatment also undergo secondary embryogenesis and this capacity has been maintained in culture for many years without diminution (Loh and Ingram, 1983, Shu and Loh, 1991). Since the embryogenic culture of winter oilseed rape is a stable system, grows well on GR-free medium and has embryogenic potential at almost all stages of the culture cycle, it is an ideal system to study the nature of totipotency and acquisition of embryogenic potential by cells without any external stimulus. Histological, electron microscopy and immunolocalisation studies were carried out to provide some insights on the morphological and cellular changes occurring in the embryogenic tissue compared to non-embryogenic tissue from the same type and at a similar stage of development. SEM and TEM studies, coupled with histological analysis revealed that the embryogenic tissue are coated by a discontinuous extracellular matrix (ECM) which forms bridges with net-like material at the onset of secondary embryogenesis and is not present in non-embryogenic tissue. However, the network became less apparent in mature embryogenic tissues particularly during growth and development of the secondary embryoids. Similar findings have been reported for maize (Samaj et al.1995), coffee (Menendez-Yuffa *et al.*, 1997) and Cichorium (Dubois *et al.*, 1991). Samaj *et al.* (1995) reported proteinaceous ECM layers and networks as possible structural markers of maize embryogenic callus. The significance of the ECM layer in winter oilseed rape embryogenic culture is not clear. Negative results of immunolocalization studies with anti-arabinogalactan protein (AGP) monoclonal antibodies (mAb) indicate that either epitopes recognized by the mAbs are not detectable/present in the ECM or it does not contain AGP at all. Immunofluorescence microscopy was carried out with JIM 4, 8, and 13 antibodies that recognize specific arabinogalactan proteins as a marker for embryogenesis (Knox *et al.*, 1989, Knox *et al.*, 1991, Pennell *et al.*, 1992). The aim was to investigate whether or not a distinct type of epidermal cell with a prominent nucleus and no marked vacuole, seen in embryogenic tissue, would show this immunological feature which has been associated with embryogenic cells (Stacey *et al.*, 1990, Toonen *et al.*, 1997, Filanova *et al.*, 2000). The analysis of immunolabelling results showed that specific epitopes were present on a limited number of cells or cell types during somatic embryogenesis of winter oilseed rape implying that these glycoproteins may have a specific role in embryogenic potential or determining the fate of the embryogenic cells. Future work is to isolate and characterize the genes that are differentially expressed between the pre-embryogenic stage in the oilseed rape embryogenic cultures and the non-embryogenic tissues of normal seedlings at a equivalent phase/point in development.

P-1435

In Vitro Plant Regeneration in *Artocarpus altilis* (Park.) Fosberg.
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Breadfruit (*Artocarpus altilis*) is the most significant food crop of the Marshall Islands of the non-coconut crop. Fruits are the most widely available starch food and regularly consumed when in season. The most common and accepted variety of breadfruit is Betaaktak, selected for the present investigation to develop know-how for the regeneration and in-vitro multiplication. Root explants obtained from the root suckers of mature tree of var. Betaaktak were grown on Murashige and Skoog (MS) medium containing Kinetin and 1-naphtalene acetic acid (NAA) in different concentration does not ensure the formation of high number of regeneration plants. A maximum of 4.2 neoplantlets per explant were obtained. After 4 week of culture on medium with benzyl adenine (11uM) and indole aceitic acid (0.5uM) multiple shoot bud induction was achieved. Incorporation of polyvinylpyrrolidone and ascorbic acid in the shoot regeneration medium was found favorable for browing in explants. More than 55% rooting in regeneration shoots was achieved when 4–5 cm individual shoots were cultured on 1/2 MS (only macro and micronutrients) medium + 2.5uM indolebutyric acid (IBA)+ sucrose (3%)+ agar (0.8%). The in vitro raised plants were successfully transferred to the soil with a success rate of 70%. In vitro plant regeneration has been reported for *Artocarpus heterophyllous* (jackfruit) but no report describe the efficient micropropagation of *Artocarpus altilis* (breadfruit).

P-1436

Normalizing Sweet Orange (*C. sinensis* (L.) Osbeck) Somatic Embryogenesis with Semi-permeable Membranes.
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Development of citrus somatic embryos initiated from embryogenic callus generally results in abnormal morphogenesis of somatic embryos. To normalize development, glycerol-induced globular stage somatic embryos of sweet orange (*C. sinensis* (L.) Osbeck cv. 'Hamlin') were cultured on 6k–8k MW cutoff cellulose acetate, >400,000k MW cutoff cellulose acetate, nitrocellulose, polyvinylidene fluoride (PVDF), cellulose filter paper, positively charged nylon, or neutrally charged nylon membranes. Only the two cellulose acetate membranes resulted in the development of normal, 2-cotyledon, bipolar, heart-shaped embryos, and no aberrant teratoma-like structures. Heart-shaped embryos could develop and germinate normally on Murashige and Tucker basal medium + 0.5% sucrose + 1 microM GA. Culture of embryogenic callus directly onto cellulose membranes also resulted in the development of normal heart-shaped embryos indicating that glycerol induction of globular stage embryos is not necessary. Heart-shaped embryos were not observed when the osmotic potential of the medium was increased by the addition of 2.5% to 15% PEG; neither were they observed when the matric potential of the medium was increased by increasing the gelling agent concentrations of agar and Gelrite from 0.8% to 3% and 0.15% to 0.9%, respectively.

P-1437

In Vitro Culture of Dewy Pine (*Drosophyllum lusitanicum*): Organogenesis and Plant Regeneration. J. NOBRE*, J. Jesus, and A. Romano. *Direcção Regional de Agricultura do Algarve, Ap. 282, 8001–904 Faro, Portugal and Universidade do Algarve, FERN, Campus de Gambelas, 8000–117 Faro, Portugal. Email: j.m.p.nobre@ip.pt

Drosophyllum lusitanicum is a rare species scarcely found in colonies at few places in Portugal, Spain and in the north most mountains of Marocco. The colonies are found on very poor and degraded soils. This species is covered with glandular red trichomes, which excrete mucilage with flytrap properties. In addition, the healing-like properties of its fresh leaves have long been recognized in folk medicine. Previous attempts to propagate this species by conventional nursery techniques failed as a result of recalcitrance of species and / or limited seed availability. A micropropagation approach is reported in the present studies. Seeds were germinated on culture medium containing MS salts supplemented with 2.22 mM BA and 2.29 mM GA_3. An heterogeneous response of the seedling explants was observed: explants forming shoots which were then multiplied and regenerated with established micropropagation protocols, and explants forming callus. Callus morphology and organogenesis where influenced both by the relative concentrations of growth regulators in the culture medium and light irradiance. Shoots were regenerated subsequently from callus cultures.

P-1438

Photoautotrophic Micropropagation Systems for Woody Plants. QUYNH T. NGUYEN[1] and Toyoki Kozai[2]. [1]Institute of Tropical Biology, NCST-VN, 1 Mac Dinh Chi Street, Hochiminh City, VIETNAM, and [2]Faculty of Horticulture, Chiba University, Matsudo, Chiba 271–8510. Email: qtnguyen@hcmc.netnam.vn, kozai@midori.h.chiba-u.ac.jp

In recent years, photoautotrophic (sugar-free medium) micropropagation has been widely applied to several plant species; which showed that the growth, development and quality of *in vitro* plantlets are strongly improved by controlling *in vitro* environmental factors to promote photosynthesis of *in vitro* plantlets. Intensive work has demonstrated the success of photoautotrophic micropropagation systems for several woody plants, such as fruit species (mangosteen), industrial plant species (coffee), and hardwood forest trees (eucalyptus, acacia, paulownia, neem, Gmelina). In the natural ventilation system, using small culture vessels attached with gas permeable filters, the growth of plantlets *in vitro* was significantly promoted when cultured on medium without sucrose, vitamins, and plant growth regulators. The increase in photosynthetic photon flux (PPF), number of air exchanges of the culture vessel and/or CO_2 concentration inside the culture vessel during the photoperiod significantly increased the net photosynthesis and the growth of coffee, acacia, eucalyptus, paulownia, neem, and Gmelina. The use of airporous supporting material such as vermiculite or Florialite (a mixture of cellulose fibers and vermiculite) enhanced the root formation of *in vitro* woody plantlets. Under the photoautotrophic condition, rooting *in vitro* observed anatomically showed a similarity to vegetative propagation as observed in the greenhouse. In the forced ventilation system using large culture vessels, a particular air mixture is flushed directly into the vessel by an air pump. CO_2 and other gaseous concentrations can be controlled by an airflow rate meter. Growth of plantlets *in vitro* of paulownia and coffee was significantly greater in the forced ventilation comparing with those in the natural ventilation.

P-1439

The Kinetics of Medium Osmolarity and Cell Wall Thickness are Good Indicators of the Onset of Embryogenesis from Cell Suspensions in Grain Legumes. S. OCHATT, F. Dhuicque, V. Ledon, and C. Schneider. INRA CR Dijon, URGAP, PCIV, BP 86510, 21065 Dijon Cedex, FRANCE. E-mail: ochatt@epoisses.inra.fr

Plant regeneration from tissue cultures of grain legumes has been reported by several groups so far, but legumes are still generally regarded as recalcitrant. In this respect, tools susceptible to distinguish regeneration-competent cells and tissues from those that will never regenerate, and as early in culture as possible, would be of interest, whatever the pathway for plant regeneration. Therefore, various parameters were examined during the culture of cell suspensions in order to identify those that might serve as early indicators of the competence of cells to undergo somatic embryogenesis. Amongst them, the time course osmolarity of the culture medium and of cell wall thickness proved both to be particularly useful, during experiments undertaken with a range of genotypes of pea (*Pisum sativum* L.) and the model species *Medicago truncatula*, and comparing different types of suspension cultured cells, including young or dense non-embryogenic and dense embryogenic cell suspension cultures. The osmolarity assessments of the culture medium (every 3–4 days during each 14-day cycle, using a vapour pressure osmometer) showed a progressive decrease in water potential preceding the onset of somatic embryogenesis, that was never observed in non-embryogenic cell suspensions, whose osmolarity increased with time in culture. On the other hand, an examination of cell wall deposition (after Calcofluor White staining and observation under UV light) and cell wall thickness (with Visilog image analyser) evidenced that cellulose accumulated in the walls with time in culture for non-embryogenic cells, whereas cell walls became thinner with the onset of embryogenesis, and diminished further as embryos matured, suggesting an increased hormonal action, concomitant with cell expansion for the embryogenic cells. The implication of these results when applying biotechnology approaches for grain legume breeding, and in terms of plant regeneration competence in general, are discussed.

P-1440

Characterization of Tissues and Regenerated Plants of Grain Legumes. S. OCHATT, C. Delaitre, F. Dhuicque, and V. Ledon. INRA CR Dijon, URGAP, PCIV, BP 86510, 21065 Dijon Cedex, FRANCE. E-mail: ochatt@epoisses.inra.fr

Difficulties have been reported for regeneration of true-to-type fertile plants from tissue cultures of grain legumes, and recent experiments in our group, with pea (*Pisum sativum*), grass pea (*Lathyrus sativus*) and the model species *Medicago truncatula* have shown that this was often linked to an abnormal nuclear DNA content. In our hands, regeneration from pea (explants and protoplasts) and grass pea (explants) proceeds mainly via organogenesis with somatic embryogenesis anecdotal while, with *M. truncatula*, embryogenesis (from explants, callus and cell suspensions) is predominant. A successful exploitation of in vitro tools to improve seed quality requires the regeneration of true-to-type plants and experiments were therefore performed with several genotypes of all species above to characterize both tissues and regenerated plants. The nuclear DNA content of each sample was assessed by flow cytometry at least twice, at monthly intervals. Whatever the species and regeneration pathway considered, a divergent phenotype was systematically linked to an abnormal nuclear DNA content. It is noteworthy that some of the genotypes studied produced only true-to-type plants (all genotypes of *M. truncatula*, LIII grass pea) irrespective of the regeneration media used, while diverging plants were observed for all pea genotypes studied as for LB and L12 grass peas. At the callus stage, some differences were also observed occasionally on *M. truncatula*, with endoreduplication the most frequent DNA content alteration, and senescence and mixoploidy less observed. Any such callus failed to regenerate or produced shoots/embryos that gave rise to infertile, non-viable plants. In turn, all tissues (calluses and regenerants) with non true-to-type flow cytometry profiles were examined further in terms of isoenzyme (EST, PER, MDH, PGM) and RAPD banding patterns compared to those of mother plants. Esterases appeared to be the best isozyme system to show differences between such materials and molecular analyses underlined these differences.

P-1441

Somatic Embryogenesis from Immature Zygotic Embryos of Annatto. V. B. Paiva Neto[1], M. N. Botelho[2], R. M. A. Euclydes[1]; E. A. M. Silva[1], and W. C. OTONI[1]. [1]Universidade Federal de Viçosa, Departamento de Biologia Vegetal/BIOAGRO, Campus Universitário, Viçosa, MG, 36571–000, and [2]Faculdade de Ciências Agrárias do Pará, Departamento de Química e Tecnologia, Pará, PA, Brasil. E-mail: wotoni@mail.ufv.br

Bixa orellana L. (Bixaceae) is a wood species known as annatto, and has become of increasing importance because of natural dyes, mainly bixin and norbixin carotenoids. Conventionally, annato is propagated by seeds, and as a cross-pollinated species it is markedly heterozygous. Therefore, the application of a reliable *in vitro* clonal propagation system would unquestionably aid in multiplication of elite types, in especial those high-producing carotenoid contents. Aiming to establish a protocol for somatic embryogenesis of annatto, immature seeds (70 days after anthesis) from adult field-grown plants, had their coats dissected out and the immature zygotic embryos removed aseptically and used as explants. The embryos were inoculated onto MS medium supplemented or not with different combinations of growth regulators and activated charcoal. Direct somatic embryogenesis was obtained in 2,4-D and/or kinetin-supplemented and non-supplemented MS medium twenty-five days after explant inoculation. The best frequencies of embryogenesis induction and embryo number were obtained with 2.26 m M 2,4-D, 4.52 m M kinetin and 1.0 g dm^{-3} activated charcoal. The presence of the latter was determinant to increase the embryo number, to reduce the time to obtain somatic embryos, and mainly to prevent callus proliferation and subsequent indirect somatic embryogenesis. However, no embryogenic response was achieved when mature seeds-derived embryos were used. It was also observed that embryogenic response was markedly affected by genotype. Histological investigation revealed that primary direct somatic embryos differentiated exclusively from protodermis or together with outer ground meristem cell layers of zygotic embryo axis, and from protodermis of zygotic embryo cotyledons. Diverse morphological differences including malformed embryos were observed among the somatic embryos. In spite of obtaining the hystodifferentiation of all embryo stages, it was observed very low conversion frequencies to normal plants from somatic embryos. FINANCIAL SUPPORT: CNPq and CAPES.

P-1442

Commercial Production of *Panax ginseng* using Bioreactor System. K. Y. PAEK and E. J. Hahn. Research Center for the Development of Advanced Horticultural Technology, Chungbuk National University, Cheongju, Chunbuk 361–763, Korea. Email: ejhahn@trut.chungbuk.ac.kr

Ginseng (*Panax ginseng* C.A. Meyer), a member of the *Araliaceae* family, is traditionally considered one of the most potent medicinal plants, having been used for centuries as a health tonic. There are many unknown active compounds in ginseng roots having anabolic, adaptogenic, antibiotic, minor hyperglycemic, and anti-cancer activities. In recent years, ginseng root culture has been successfully applied to the production of useful secondary metabolites, including pharmaceuticals, pigments, and other fine chemicals. On the other hand, a large-scale production system is required to increase the productivity. In this regard, bioreactor culture has many advantages over the conventional culture. It is easy to control chemical and physical environments such as the composition and supply of culture medium, the number of air exchanges, and the treatment of elicitors to optimize the culture conditions for the root growth and for the secondary metabolite production. We investigated the growth of ginseng roots and the production of secondary metabolites under various environments to determine the optimal culture condition using small-scale bioreactors. Based on the results, large-scale bioreactor cultures (500–1,000 liter) of ginseng roots were applied, from which 4 to 8 kg of root dry weight was achived. The cultured ginseng root has been transferred to commercial companies to use as a raw material of cosmetic and pharmaceutical products.

P-1443

Transgenic Rice Production via WHISKERS™-mediated Transformation of Embryogenic Callus. D. PAREDDY, M. Lu, T. Strange, B. Waldman, V. Patterson, M. Skokut, and J. Petolino. Dow AgroSciences, Indianapolis, IN 46268. E-mail: drpareddy@dowagro.com

The delivery of DNA into plant cells via silicon carbide WHISKERS™ offers a simple and inexpensive method of plant transformation. In fact, WHISKERS™ technology has been used for routine and large-scale transgenic production in maize (Bullock et al., 2001). However, this has required the use of embryogenic suspension cultures. Somaclonal variation limiting fertility and seed production, and genotype specificity are some of the technical problems usually associated with suspension cultures. Although soft, friable, 'Type II' embryogenic callus of maize has been transformed using WHISKERS™ (Petolino et al., 2000), this type of callus is limited to only a few genotypes of maize and oats. In general, the cereal and grass species produce hard, compact embryogenic callus, which is referred to as 'Type I' callus. Here, we report transgenic rice production via WHISKERS™-mediated transformation of compact embryogenic callus. Transient GUS expression and/or stable transformation was used to optimize various parameters including osmoticum treatment, agitation method, and multiple inoculation. Stable transgenic plants were produced using both the *hpt* and *pat* selectable markers. On average, 20–30% transformation frequency (To plant events on a 'per 250 mg sample' basis) was obtained following hygromycin selection. Such frequencies of transformation allow the use of WHISKERS™ technology as an alternative and reliable method for the production of transgenic rice. Also, considering transformation of compact, 'Type I' embryogenic callus of rice, WHISKERS™ technology could potentially be applied for transformation of other monocot species such as maize, wheat, barley, etc. without the need for initiation of suspension cultures.

P-1444

In Vitro Collecting: Response of Leaf Tissue from Four Sites to Antibiotics and Antioxidants. VALERIE C. PENCE. Center for Conservation and Research of Endangered Wildlife, Cincinnati Zoo and Botanical Garden, 3400 Vine Street, Cincinnati, OH 45220. E-mail: vcpence@aol.com

Previous studies have indicated the potential usefulness of in vitro collecting (IVC) leaf tissue as a readily available source of germplasm when seeds are not available. Controlling contamination and initiating growth are two critical factors in successful IVC. To further improve this technique, the effectiveness of antimicrobial and antioxidant treatments was compared with 77 species from sun and shade in Utah, Arizona, Ohio, and Florida. Young leaf tissues were surface sterilized in 70% ethanol and transferred aseptically to vials of MS medium with 0.5 mg/L BAP, 0.5 mg/L NAA, and 100 mg/L benlate (fungicide) with either antibiotics (5 mg/L cefotaxime and 0.25 mg/L vancomycin, dissolved and sterilized immediately before adding .05 ml on top of tissue), 0.25 ml/L PPM(r) (added to the medium before autoclaving), or both antibiotics and PPM. Antioxidants were added to half the media, as 1 mg/L citric acid and 500 mg/L PVP. After 2–3 weeks, tissues were evaluated for contamination, and clean tissues were transferred to several media for further growth. The percent of tissues remaining free of visible bacteria or fungi within two weeks of collecting was greater than 90% for all four sites. There were no consistent differences between PPM and the antibiotics in controlling bacteria, although PPM may have provided some additional control of fungus. Fungus was higher for humid areas generally, while tissue browning was higher from arid areas. There were differences in the responses of sun and shade plants, depending on the habitat. There appeared to be no effect of the antioxidants either on tissue browning or on any other parameter measured. The percent of tissues showing growth was higher from arid rather than humid areas, with 88–95% of species forming callus and 20–40% forming buds, depending on the site. These results provide further information on the effects of habitat on IVC and suggest approaches for future improvements of the technique.

P-1445

Virus Free and Mass Propagation of Pitahaya *Hylocereus triangularis* L. M. PEREA DALLOS, J. Rodríguez Riaño, and A. Tirado Perea. Departamento de Biología, Universidad Nacional de Colombia, P.O. Box 14490, Bogotá – Colombia. E-mail: mapere@ibun.unal.edu.co, andretirado@yahoo.com

The Pitahaya belongs to the climbing cacti family, and epiphyte group native to the forest of northern South America and Central America. The most important species is *Hylocereus triangularis* L. which produces a yellow, soft, sweet and delicious fruit. Studies on Pitahaya have been carried out specially on phosphorus and ascorbic acid. Recently, cactine was isolated, this compound has cardiotonic effects.This crop is propagated by seeds and stems, however there is a hight risk to spread viral disease of which has been reported. For this reason, attempts to produce virus free plants by means of shoot-tip is under taken. Additionally, a rapid micropropagation system have been developed. Our studies have been directed to genetic stability for mass propagation through meristematic structures using Murashige and Skoog medium (M&S, 1962) with 30g/L sucrose, pH 5.8, 2% Gelrite(r), supplemented with 6-benzylaminopurine (BAP) 0–05mg/L, and 0–0.5mg/L of Naphtalene acetic acid (NAA). Culture were transferred at 27C ± 1 with 16/8 as photoperiod. After 10 weeks we observed 10 – 15 shoot explants. For rooting, shoots of 2cm size were subcultured in the basal M&S medium with 0.5 – 1.0 mg/L of NAA, after 6–8 days roots gave a complete plant. For breeding purposes this research is in progress in order to get resistence plants to fungus diseases.

P-1446

Direct Somatic Embryogenesis and Plant Regeneration in *Epidendrum rhuizianum*. M. PEREA DALLOS and J. M. Pelaéz Montes. Departamento de Biología, Universidad Nacional de Colombia, P.O. Box 14490, Bogotá-Colombia. E-mail: mapere@ibun.unal.edu.co, jpelaezmontes@yahoo.es

Colombia is the third country in the world for flowers exports. Orchids is one of the groups has the great diversity in different zones and also one with high risk of extinction, because of the beauty of its flowers, our country has an important place for the orchids market. Plant propagation is a good alternative in order to obtain numerous plants to establish commercial crops and improvement for new interesting hybrids. In this research we found the way for mass propagation through somatic embryogenesis, which represents the first work in orchids in this subject in our country. We choosed *Epidendrum rhuizianum* to be a specie very exotic and unique in the Andean zones. Capsules of this orchid were transferred to our laboratory and its seeds placed under aseptics conditions using Murashige and Skoog medium (M&S) for its development. The plantlets obtain after 9 months of incubation were transferred in M&S medium supplemented with agar-agar Merck(r) 8g/L, 2,4 –diclorophenoxyacetic acid (2,4-D) 0–3 mg/L and Thiadiazuron (TDZ) 0–3 mg/L alone or in combinations, with pH 5.8 at 27°C±1 in the dark for 10 weeks. Once the globulars structures were observed, the explants were placed in flasks with M&S medium, with agar-agar Merck(r) 8g/L, without growth regulators, with pH 5.8 for embryos ripe, incubated at 27°C±1under photoperiod of 16 hours light during 8–10 weeks. In this research we founded that direct somatic embryogenesis is the main way of plant regeneration in this orchid, obtained about 150–200 plants per flask.

P-1447

Reproducible *Agrobacterium*-mediated Transformation of Barley (*Hordeum vulgare* L.) for Clean Gene Technology. MATTHEW D. PERRY, Shona Ross, Silvia Travella, Judith Harden, Vera Browne, John W. Snape, & Wendy A. Harwood. Crop Genetics Department, John Innes Centre, Norwich Research Park, Norwich, NR4 7UH, U.K. E-mail: matthew.perry@bbsrc.ac.uk

The reproducible, *Agrobacterium*-mediated, transformation of cereal crops, is key to the application of GM technology within commercial agriculture. Unlike direct transformation methods, such as particle bombardment, *Agrobacterium*-mediated transformation often results in the integration of undisrupted transgenes, at low copy number (typically 1 or 2). The development of binary vectors containing two independent T-DNAs permits the application of clean gene technology, in which selectable marker genes may be removed from the final transgenic plants by a simple backcross. The removal of unwanted marker genes facilitates subsequent gene pyramiding and, addresses consumer and legislative concerns regarding the development of GM technology. Existing protocols for the *Agrobacterium*-mediated transformation of barley have been both difficult to reproduce and awkward to transfer between laboratories; A series of independent investigations, over a period of 2 years, using a variety of selectable markers and reporter genes is described. In excess of 145 transgenic plants were generated, including 35 independent lines.

P-1448

Callus Formation and Plant Regeneration from *Baccharis trimera* (Less) D. C., a Medicinal Plant. JOSÉ EDUARO PINTO, Fabiano G. Silva, and Suzan K. Bertolucci. Department of Agriculture, University of Lavras, Minas Gerais, Brazil 37200–000. Email: jeduardo@ufla.br

Baccharis trimera (Less) D. C. belonging to the Family Asteraceae (Compositae), also known as "carqueja", is a popular medicinal plant in Brazil used in folk medicine as a tonic diuretic; anti-diabetic agent; cholesterol lowering properties. Callus culture were established from stem tissue of *Baccharis trimera* on Murashige and Skoog (MS) basal medium and half strength of salts, vitamins and inositol. Both medium were supplemented with different balances (15 total) of plant growth regulators (BAP, kinetin, and NAA) in the concentration of 0.0, 2.5, and 10.0μM. In the second experiment, two cytokinins (Kinetin and TDZ) at the concentration of 0.0, 1.25, and 2.5μM and two auxins (Naa and 2,4-D) at the concentration of 0.0; 5.00; 10.0; and 15.0μM were evaluated (totaling 35 balances of growth regulators). In the first experiment, after 60 days were verified through the characteristics of fresh and dry biomass that callus development was improved by using lower medium slats, vitamins and inositol concentrations. Highest callus formation was observed on a medium containing NAA under 15μMol.$m^{-2}.s^{-1}$ light intensity with a 16-h photoperiod. Among the cytokinins, kinetin was more efficient than BAP for callus induction. In a second experiment, at the end of 45 days, auxins proved to be more efficient in the induction and growth of callus than cytokinins and among auxins studied NAA 15.0μM was the efficient than 2,4-D. The highest rate of in vitro shoot regeneration was obtained by using 2.50μM of thidiazuron (TDZ) as growth regulator. Rooting was achieved by culturing the shoots directly on MS medium.

P-1449

A Histological and Biochemical Study of Avocado Zygotic Embryogenesis. F. PLIEGO-ALFARO[2], R. Perán Quesada[1], C. Sanchez-Romero[1], B. Marquez-Martin[1], A. Barceló-Muñoz[1]. (1) Centro de Investigación y Formación Agraria, 29140 Churriana, Málaga and (2) Dpto. De Biología Vegetal, Facultad de Ciencias, 29071 Málaga, Spain. E-mail: ferpliego@uma.es

Somatic embryogenesis offers potential advantages to make effective the utilization of biotechnological approaches such as genetic transformation. However, the final performance of this *in vitro* regeneration method is largely affected by the extent to which the pattern of somatic embryo development matches that of zygotic embryogenesis.The aim of this investigation was to establish a model of embryogenic development in avocado by monitoring growth, histochemical and biochemical parameters along zygotic embryo development. Variations observed were correlated with a real criterion of maturity: germination capacity under standard *in vitro* culture conditions. Defining specific features or markers characterizing the different embryogenic phases, would allow to determine the way in which nutrients, hormones and other culture factors affect embryogenesis *in vitro*. Cell division and histodifferentiation were characterized by high hexoses levels, elevated hexoses/sucrose ratio and intense RNA staining. Both phases were differentiated morphologically by histochemical techniques. Histodifferentiation was accomplished at relatively early developmental stages and the subsequent decrease in mitotic activity, was revealed by a drastic decline in RNA staining. After a short transition phase, the beginning of maturation was revealed by a significant increase in fresh and dry weights and a decrease in water content and hexoses/sucrose ratio. Starch content increased and protein bodies were visualized for the first time. At the end of the reserve products deposition, embryos presented maximum fresh and dry weights, minimum water content, the highest levels of sucrose and starch and the lowest of hexoses. Starch granules and protein bodies appeared profusely. The complete maturation stage, at which a 100% of germination was achieved, could be identified by the synthesis and accumulation of a specific storage protein of 49 kD.

P-1450

Somatic Embryogenesis-specific Arabinogalactan-proteins in Cotton Cell Cultures. S. POON, R. L. Heath, and A. E. Clarke. Plant Cell Biology Research Centre, School of Botany, University of Melbourne, Vic. 3101, Australia. E-mail: simonp@unimelb.edu.au

One of the major bottlenecks in the production of transgenic cotton is the difficulty in regenerating whole plants from elite cell culture. Embryogenic potential in cotton is highly genotype-dependent and despite persistent efforts, somatic embryogenesis in cotton cells occurs at a low frequency or not at all. Of the cultivars found to be regenerable, most are no longer grown commercially. To introduce genes into elite cultivars, a regenerable cultivar is transformed and then entered into a backcross breeding program. This process delays the commercial release of genetically engineered cotton by several years. Recently, a class of plant proteoglycans known as arabinogalactan-proteins (AGPs) has been found to promote somatic embryogenesis in species such as carrot and spruce. The mode of action and the identities of these AGPs are unclear. Moreover, despite their abundance and wide distribution in the plant kingdom, there is no conclusive evidence for the role(s) of AGPs in plants. We have isolated and characterized several AGPs that are specifically expressed by embryogenic cotton cell cultures. We are attempting to understand the mode of action of these molecules and we are investigating whether they can be applied or manipulated to promote somatic embryogenesis in elite cotton cultivars.

P-1451

The Effect of Exogenous Cytokinins on the Levels of Endogenous Cytokinins During Induction of Organogenesis on Tobacco Leaves In Vitro. M. Klemš, P. Krejci, V. Reinöhl, and S. PROCHÁZKA. Department of Botany and Plant Physiology, Faculty of Agronomy, Mendel University of Agriculture and Forestry Brno, Zemědělská 1, Brno 613 00, Czech Republic. Email: botanica@mendelu.cz

The effect of exogenous cytokinins (6-benzylaminouine – BAP or Isopentenyladenin – iPA) taken up during 20 hours of induction, on the levels of endogenous cytokinins (bases, ribosides, ribotides, and glucosides) and on direct organogenesis on tobacco leaves of cv. Petit Havana SR1 transformed with cytokinin-glucoside specific β-glucosidase Zmp 60.1 (SR1-Z) and cv. Wisconsin 38 (W38) was investigated. Different times of shoot organogenesis after subsequent 3–5 weeks of culture on MS medium were observed in these two tobacco types. Tobacco leave explants on induction medium with an auxin: cytokinin ratio 1.6: 1 (2,4-D 0.8 $mg.l^{-1}$, BAP or iPA 0,5 $mg.l^{-1}$) intensively took up and metabolized the exogenous cytokinins (predominantly to 7-glucosides and also to 9-riboside monophosphates) and the content of endogenous cytokinins decreased (iPA and Z and their metabolites on medium with BAP, and BAP and Z and their metabolites on medium with iPA) Twenty-four hours after transfer to a hormone free MS medium, the content of BAP and its conjugates in BAP-induced explants was still increasing, while the content of iPA and its conjugates in iPA-induced explants remained unchanged. It is possible that in iPA-induced explants, the iPA biosynthesis also increased, and simultaneously the degradation by cytokinin oxidase (*CKX*) was more intensive. Only a longer period of measurement of endogenous CK levels and changes of *CKX* activity will clear the question if a habituation process in tobacco leaves has been established.
This work was supported by the project MSM 43210001.

P-1452

Development of Large and Opaque Avocado Somatic Embryos: Effects of Culture Age, Desiccation, and Genotype. S. H. T. RAHARJO and R. E. Litz. Tropical Research and Education Center, IFAS, University of Florida, 18905 SW 280 St., Homestead, FL 33031–3314. Email: srajarjo@mail.ifas.ufl.edu

Proembryonic masses and globular somatic embryos of various genotypes of avocado were established and routinely maintained in liquid modified MS medium containing 0.41 μM picloram; however, formation of fully mature (>5 cm) bipolar somatic embryos was sporadic. To achieve optimal conditions prior to and during somatic embryo formation, age of culture before plating (10, 25, 20, 25, and 30 days) and desiccation treatments (transfer onto filter paper in Petri dishes without medium for 0, 2, 6, 10 days) were tested, using several culture lines, including Topa-topa, Suardia 1.1, Reed 5.2, Fuerte 3.2 CG (Transformed with chitinase-glucanase genes) and pHass 3.7 AFP (transformed with anti-fungal protein gene). Large (>0.5 cm) and opaque somatic embryos (1 to 26 per 90 x 10mm Petri dish) developed in Topa-topa, Suardia 1.1, Reed 5.2, and pHass 3.7 AFP on MS medium with 20% (v/v) liquid coconut endosperm and 6 g/l gellan gum. Optimum conditions for recovery of mature somatic embryos included: 1) 15-day-old cultures (after last subculture); 2) a desiccation treatment for 2 days prior to plating; and 3) the culture dishes were not sealed with Parafilm.

P-1453

Molecular Genetic Analysis of the Two Defective Embryo and Meristems (DEM) Genes in *Arabidopsis*. C. M. RAMAGE[1,2], C. Brosnan[2], L. Matthew[2], M. E. C. Reyes[2], and B. J. Carroll[2]. [1]School of Agriculture and Horticulture, University of Queensland-Gatton, Gatton 4345, Queensland, Australia and [2]Department of Biochemistry and Molecular Biology and School of Land and Food Sciences, University of Queensland, Brisbane 4072, Queensland, Australia. E-mail: ramage@biosci.uq.edu.au

The *Defective embryo and meristems* (*Dem*) gene first cloned from tomato is expressed in all of the major sites of plant differentiation, namely in the embryo and meristems (Keddie *et al.*,1998). Stable tomato *dem* mutants lack functional meristems and fail to develop beyond the seedling stage, but appeared to have a normal hypocotyl. A somatic mutagenesis line was used to demonstrate that the tomato *Dem* gene is required for formation of the leaf blade, and the terminal differentiation of palisade and adaxial epidermal cells (i.e. adaxial leaf tissue). However, the *Dem* gene was not required for differentiation of abaxial leaf tissue in tomato. BLAST searches indicated that two *Dem*-like genes exist in *Arabidopsis*. The molecular genetic analysis of two *Arabidopsis Dem*-like genes will be reported. Intron-spliced dsRNA knockouts of the two genes gave distinct phenotypes in the embryo. Using promoter fusions to GFP and GUS marker genes and Northern analysis, the two genes showed differential tissue-specific expression in post-embryonic tissues. The results indicate that the two *Arabidopsis Dem*-like genes play crucial and complementary roles in plant development. The precise biochemical function of DEM-like proteins has not been determined but a two-hybrid screen of *Arabidopsis* cDNA libraries has identified candidate proteins for interaction with DEM-like proteins. 1. Keddie, J. S. *et al.* (1998) Plant Cell 10, 877–888. 2. Smith, N. *et al.* (2000) Nature 407, 319–320.

P-1454

In Vitro Propagation of *Saintpaulia rupicola*, an Endangered Species of African Violet. MICHAEL H. RENFROE and Evonne N. Johnson. Dept. of Biology, James Madison University, Harrisonburg, VA 22807. Email: renfromh@jmu.edu

African violets are among the most endangered plant species. In Vitro propagation may offer a means of maintaining germplasm stocks of some of these species. We developed a method for in vitro propagation of *Saintpaulia rupicola*, an endangered species of African Violet native to Kenya. Axenic leaf explants were transferred onto media containing various combinations of BAP and NAA. Greatest regeneration was obtained on media containing BAP at 2.5 mg/l with NAA at 0.25 mg/l or 0.05 mg/l, and on medium with BAP at 0.25 mg/l and NAA at 0.05 mg/l. A brown discoloration developed in several of the media. Additional experimentation was conducted to test the effects of inclusion of 0.1 g/l ascorbic acid, 0.1 g/l PVP, or 0.1 g/l activated charcoal. Leaf sections from three genotypes were placed on the media and observed for browning. Genotypic differences were observed in media discoloration. Antioxidant additives reduced browning slightly. Regenerated shoots were isolated, rooted in vitro, and transferred to soil in pots. Plants were acclimatized to ambient conditions and continued to grow.

P-1455

Calli Induction and Adventitious Shoot Regeneration from Leaves of Carob Tree (*Ceratonia siliqua* L.). Micropropagated Plants. L. CUSTÓDIO, P. Jesus, & A. Romano. Faculdade de Engenharia de Recursos Naturais, Universidade do Algarve, Campus de Gambelas, 8000–117 Faro, Portugal. E-mail: aromano@ualg.pt

The purpose of this work was the development of a suitable adventitious regeneration system for *Ceratonia siliqua* L. starting from leaves of micropropagated plants. The influence of different factors affecting *calli* induction and adventitious shoot regeneration from leaflets of two cvs. of carob tree ('Mulata' and 'Galhosa'), were studied: auxin/citokinin rate, presence or absence of light, age of the plant material and explant type. On cv. 'Mulata' *calli* production was induced in all growth-regulator treatments. In this cultivar dark incubation during the first 20 days of induction did not improve calogenesis. On cv. 'Galhosa' the frequency of *calli* formation was lower, and the incubation of leaflets in darkness improved calogenesis results, only when the induction media contained TDZ. Explants from micropropagated plants growing in the glasshouse lead to higher calogenesis formation than those from *in vitro* cultures. Trials with the basal portion of leaflets conduced to higher *calli* formation. Shoot regeneration occurred via an intervening *callus* phase in induction media containing 5 $mg.l^{-1}$ BA + 1 $mg.l^{-1}$ ANA, 5 $mg.l^{-1}$ zeatina + 0.1 $mg.l^{-1}$ ANA, 1 $mg.l^{-1}$ TDZ + 1 $mg.l^{-1}$ ANA, 1 $mg.l^{-1}$ TDZ + 0.01 $mg.l^{-1}$ AIA and 5 $mg.l^{-1}$ TDZ + 0.01 $mg.l^{-1}$ 2,4-D. These shoots were obtained in the non-basal portion of the leaflets, on trials in the light and either in induction or elongation media. The multiplication rate of the regenerated shoots was similar for both cultivars.

P-1456

In Vitro Germination and Early Growth of Seedlings of Two Species of *Bletia* (Orchidaceae). S. LUNA-ROSALES, A. Barba-Alvarez, and R. Perez-Cruz. Unidad de Investigación en Biología Vegetal, Facultad de Estudios Superiores-Zaragoza, UNAM. AP 0920, CP09230, México,D.F. E-mail: BARBARAL@SERVIDOR.UNAM.MX

The asymbiotic germination and subsequent development of seeds of two Mexican terrestrial orchids *Bletia purpurata and B. macristhmochila* were studied using two basal media, Knudson C (KC) and Mitra (M), and supplemented with casein acid hydrolisate (CH) or yeast extract (YE) as organic compounds. Seeds germinated for all treatments. Seed germination of *B. purpurata* was 100% using KC, M and M+CH media, whereas *B. macristhmochila* only with KC medium. Some development, ranging from embryo swelling to shoot and root development, was observed for the two species. After three months of *in vitro* culture only on KC medium more than 50% of seedlings developed roots. This study further demonstrates that the response of *Bletia* seeds to different media during germination and development varies with species.

P-1457

Asymbiotic and Symbiotic Germination and Growth of *Bletia gracilis* (Orchidaceae). S. LUNA-ROSALES, A. Barba-Alvarez, F. Garcia-Suarez, and D. Serrano-Hernández. Unidad de Investigación en Biología Vegetal, Facultad de Estudios Superiores-Zaragoza (Campo II), UNAM, AP 0920, CP 09230, México, D.F. E-mail: BARBARAL@SERVIDOR.UNAM.MX

Asymbiotic and symbiotic germination of seeds of a Mexican terrestrial orchid *Bletia gracilis* were tested. Seeds of *B. gracilis* were germinated *in vitro* on Knudson C medium supplemented with coconut water (KC+CW) and Basic Oat medium inoculated with an endophyte (O+) isolated from roots of a naturally occurring plant of the same species. Germination was high with both asymbiotic and symbiotic methods. Seedlings grew from green protocorms to plantlets with different degrees of shoot and root formation. Significantly higher development rate was found on asymbiotic culture.

P-1458

Transgenic Tea (*Camellia sinensis* (L.) O. Kuntze). INDRA SANDAL, Amita Bhattacharya, Sanjay Kumar, and Paramvir Singh Ahuja. Institute of Himalayan Bioresource Technology, Palampur-176061, H. P, India. E-mail: director@csihbt.ren.nic.in

Tea (*Camellia sinensis* (L.) O. Kuntze) is an important non alcoholic beverage drink that has become very popular due its nutritional, anti-oxidative, anti-microbial and anti-cancerous properties. Although tea is an important commercial crop for many Asian countries, considerable loss is incurred due to diseases and other biotic and abiotic stresses. Being a woody perennial crop with a long life cycle of 10 years, tea requires improvement through transgenic technology. A highly efficient but reproducible regeneration system was developed via callus phase through sequesteration of 2,4-D from leaf explants. This regeneration system was then employed for the first time for production of biolistic mediated transgenic tea. Different parameters involving 360 combinations and permutations of distances (TD, GD, MFD) coupled with factors viz. burst pressure, concentration of DNA and osmotica were optimized for 100% GUS expression when pRT99GUS was used. While distances, burst pressure and concentration of DNA were found to be important factors, stable transformation was improved significantly when the leaf explants were precultured in 0.25 M sorbitol or hormone free medium. About 40% of the transformants that survived the lethal dose of kanamycin showed PCR amplification for both nptII and gus primers. The results of southern hybridization further confirmed the stable transformation in 40% of the kanamycin selected transformed micro-shoots that were micrografted on seedling raised root stocks and hardened in CO_2 enriched chambers with overhead lights. The evolved regeneration-transformation protocol is now being employed for the introduction of genes of interest for disease resistance and stress adaptations.

P-1459

The Acid Growth Model and pH-directed Triple Functions in Embryogenic Cell Suspension Culture of Banana Musa AAA and AAB Genomic Groups. J. P. Chung, C. C. Lu, C. T. SHII. Department of Horticulture, National Taiwan University, 106 Taipei, Taiwan, ROC. E-mail: shiict@ccms.ntu.edu.tw

The extracellular pH in embryogenic cell suspension culture of triple bananas can be auto-regulated at 3.3–4.0, 4.0–4.6 and 4.6–5.3 under the same TB5 maintenance medium, is incarnated in growth triple functions of multiplication, proliferation and globularization phases. In regardless of genomic group, auxin depletion, culture volume or subculture interval, the auto-regulated pH steady state is specific to growth phase suggests to be the developmental regulation. It was also demonstrated that the growth phases are reversible and simulate through the sole pH control or pH related additives. Simulating pH 3.50 ± 0.20 culture conditions, both preembryogenic masses (PEMs) of proliferation and globularization phases are inducible to multiplication phase active in releasing cell propagules. The free preembryogenic determined cells (PEDCs) dominate in symmetric division with 1.07 ± 0.07 size ratio of two daughter cells. Upgrading to pH 4.40 ± 0.20, the free PEDCs are more active in symmetric division and formation of amorphic PEMs. In contrast, controlling external pH at 4.95 ± 0.20, the PEDCs of either multiplication or proliferation phases are directed to embryogenic determined cells (EDCs) which form unequal division with 1.31 ± 0.16 size ratio of 81% two daughter cells in 48 hrs, and achieves 68.4% T-shaped tri-cells by the fourth day. The destination of large apical cell develops to octant stage of proembryo is traceable. The PEDCs is also directed to proembryogenesis while adding 1.6–2.2 mM NaH_2PO_4 is approachable to pH 4.6–5.3. The acid growth model is suggested that the auto-regulated proton influx or controlling external pH over the threshold pH 4.6 may serve as polar axis signal transforming PEDC to EDC destining to proembryogenesis, however proton efflux or controlling pH below the threshold may act as cell polar axis eliminator and cell releasing result in apolar growth. The manipulation of pH-directed triple functions to achieve homogenous PEDCs or EDCs is useful in diverse development of banana biotechnology.

P-1460

The Use of Sugarcane Leaf Roll Discs as Target Material and Regeneration of Transgenic Plants via Direct Embryogenesis: Problems And Potential. SANDRA J. SNYMAN[1,2], Barbara I. Huckett[1,2,3], Frikkie C. Botha[2], and M. Paula Watt[3]. [1]South African Sugar Association Experiment Station, P/B X02, Mount Edgecombe, 4300; [2]Institute for Plant Biotechnology, University of Stellenbosch, P/B X1, Matieland, 7602; and [3]School of Life and Environmental Sciences, University of Natal, Durban, 4041 South Africa. E-mail: snyman@sugar.org.za

The use of meristematic leaf roll discs as target material for gene delivery by particle bombardment is a novel approach in sugarcane transformation. This pathway of morphogenesis offers potential advantages in terms of efficiency and, due to low levels of auxin, also offers the potential advantage of genetic stability. Three routes of transformation were compared: (1) bombardment of leaf discs, regenerated via direct embryogenesis on 0.3 mg/l 2,4-D, (2) bombardment of leaf discs, regenerated via indirect embryogenesis on 3mg/l 2,4-D and (3) standard bombardment of embryogenic callus initiated and maintained on 3mg/l 2,4-D. Plant regeneration on low stringency selection medium took 13–22 weeks (route 1) or 18–26 weeks (route 2), compared to 24–36 weeks (standard; route 3). PCR, Southern and phenotypic analysis indicated that, regardless of the regeneration route, the majority of plants were escapes. As the transgene and specific phenotype in half of the plants regenerated via direct embryogenesis (route 1) were detectable only in young plants; the effects disappearing with further growth and development, it was hypothesised that these plants were chimaeric. Optimisation of the timing of gene delivery and the selection regime will be necessary if this route is to be used in a transformation system. When the selection pressure was increased, a transformed line was obtained via route 1. However, greatest promise is shown by route (2) which, in addition to being faster, demonstrated a stable transformation frequency 2.5 times higher than that observed in the conventional protocol (route 3).

P-1461

Factors Affecting Transformation of *Brassica napus* Using *Agrobacterium tumefaciens*: Genotype and *Agrobacterium* Strain. K. SONNTAG. Federal Centre for Breeding Research on Cultivated Plants, Institute of Agricultural Plants, Rudolf-Schick-Platz 3a, 18190 Groß Lüsewitz, Germany. E-mail: k.sonntag@bafz.de

Transformation efficiency is influenced by many factors, including cultivar type of explant conditions for plant regeneration, selection and *Agrobacterium* strain. *Agrobacterium*-mediated transformation of *B. napus* has not been without problems as rapeseed appears to be variable in its response to *Agrobacterium spp*. Hypocotyl explants were inoculated with disarmed *A. tumefaciens* strains ATHV C58C1 and GV3101, and then cultured on media containing kanamycin. The *A. tumefaciens* strains harbored a binary vector, which contained a neomycin phosphotransferase II (NPT II) gene and seed oil genes driven by the 35S promoter of cauliflower mosaic virus and different promoters (FatB4, napin), respectively. Transformation of *B. napus* plants was confirmed by detection of NPTII enzyme activity. *Brassica napus* L. cv. 'Drakkar' and two breeding lines were used for the comparison of genotypes. Variable transformation frequencies were observed with different types of plant material. Reduced regeneration and transformation effiency was obtained with three constructs with 'Drakkar'. The difference between 'Drakkar' and the breeding line was up to 10% in the production of transgenic plants. Plant transformation with two different *Agrobacterium* strains revealed significant difference in the capability to regenerate shoots although they both belonged to the nopaline type. Use of the hypervirulent strain of *A. tumefaciens* ATHV C58C1 consistently produced an approximately twofold higher transformation response. These results were obtained in a cooperative project with the University of Hamburg (E. HEINZ, H. SCHMIDT, P. SPIEKERMANN) and the Breeding Company NPZ (M. FRAUEN).

P-1462

The Effect of Polyethylene Glycol (PEG) on Gene Expression of Developing White Spruce Somatic Embryos. CLAUDIO STASOLLA, D. Craig, Z. Li, L. van Zyl, and R. R. Sederoff. Forest Biotechnology Group, North Carolina State University, Raleigh, NC 26795. E-mail: cstasol@unity.ncsu.edu

White spruce somatic embryogenesis has been extensively utilized as a model system for investigating many physiological and molecular aspects of embryo development in coniferous species. Methods for improving the development of somatic embryos involve manipulation of the culture conditions, such as inclusions of abscisic acid (ABA) and osmoticum into the maturation medium. In recent years, applications of polyethylene glycol (PEG), a non-permeating osmoticum agent, have been proven to increase the number of embryos produced in vitro, as well as their quality. In general, PEG-treated embryos have an increased tolerance to drying, and accumulate a large amount of storage products, similar in composition to those observed in their zygotic counterparts. In order to elucidate the molecular mechanisms underlying these processes, the expression analysis of more than 2000 expressed sequence tags (ESTs), many of them similar in sequence to *Arabidopsis* genes involved in embryo development, was conducted. Our results demonstrated that substantial differences in gene expression occur during the embryo maturation process, as well as between control and PEG-treated embryos. These data represent valuable information for the design of new experiments aimed at improving the quality of somatic embryos.

P-1463

Pyrimidine Metabolism During Shoot Organogenesis. CLAUDIO STASOLLA, N. Loukanina, H. Ashihara*, E. C. Yeung, and T. A. Thorpe. Department of Biological Sciences, University of Calgary, Calgary, Alberta Canada, T2N 1N4 and *Department of Biology, Ochanomizu University, Tokyo, Japan, 112–8610. E-mail: cstasol@unity.ncsu.edu

Changes in the pattern of pyrimidine nucleotide metabolism were investigated in *Pinus radiata* cotyledons cultured under shoot-forming (SF) and non shoot-forming (NSF) conditions, by following the metabolic fate of externally supplied ^{14}C-labeled orotic acid, an intermediate of the de novo pathway, and ^{14}C-labeled uridine and uracil, substrates of the salvage pathway, as well as by measuring the activity of key enzymes. The de novo synthesis of pyrimidine nucleotides was operative under both SF and NSF conditions, but the activity of orotate phosphoribosyltransferase (OPRT), a key enzyme of the de novo pathway, was higher in SF tissue. Salvage synthesis of nucleotides was also operative in SF and NSF cotyledons, as both uridine and uracil were utilized for nucleotide and nucleic acid synthesis. Utilization of uracil for nucleic acid synthesis was lower in NSF cotyledons, compared to that observed for SF tissue after 10 days in culture, due to higher uracil phosphoribosyltransferase (UPRT) activity in the latter. Throughout the culture period, uridine was a better substrate for the synthesis of salvage products than uracil; possibly due to the higher activity of uridine kinase (UK), compared to uracil phosphoribosyl-transferase (UPRT). Thus, it appears that the increased ability to produce pyrimidine nucleotides via the salvage pathway may represent a metabolic switch required for the initiation and subsequent development of shoot bud primordia in *Pinus radiata*.

P-1464

Direct Somatic Embryogenesis in Pepper. B. STEINITZ[1], M. Küsek[2], Y. Tabib[1], I. Paran[1], and A. Zelcer[1]. [1]Department of Plant Genetics, Agricultural Research Organization, The Volcani Center, Bet Dagan 50250, Israel, and [2]Dept. of Plant Protection, Faculty of Agriculture, University of Kahramanmaras Sutcu Imam, 46060 Kahramanmaras, Turkey. E-mail: steinitz@netvision.net.il

Pepper (*Capsicum annuum* L.) is considered a recalcitrant species in terms of plant regeneration *in vitro*. For several decades research efforts have been focused mainly on regeneration via organogenesis whilst reports on somatic embryogenesis dealt mainly with the regeneration of dihaploids in anther culture. Studies considering somatic embryogenesis from vegetative tissue other than anthers started to appear only in recent years and the information available is limited as yet. Our objective was to explore the possibility to regenerate plants by somatic embryogenesis in sweet, pungent, and paprika types, using seed-derived zygotic embryos as source explant. After identification of several basic culture parameters important to obtain reproducible results, we examined the role of plant growth regulators in embryogenesis induction and in embryo maturation and conversion to plants. Facile direct somatic embryogenesis was induced in different genotypes by chlorophenoxy acids supplemented to the medium. Cytokinin supplement was found to be unnecessary for direct embryogenesis induction; moreover, it promoted callus formation, which was detrimental to embryogenesis. We will describe the dose response relationships for embryogenesis induction by different auxins and the time course of the appearance of somatic embryos on the explants, and we will present photomicrographs depicting embryo morphogenesis. Since embryos were generally morphologically aberrant and failed to develop into normal plants, we shall discuss at which stages in embryo development the major anomalies become established.

P-1465

Propagation of Blue Flowered *Conospermum* Species. L. STONE[1], J. McComb[1], and K. Seaton[2]. [1]Biological Sciences, Murdoch University, Murdoch, WA 6150, and [2]Department of Agriculture Western Australia, South Perth, WA 6151. E-mail: lstone@central.murdoch.edu.au

The increasing demand for Australian native flowers on the export market has prompted a study into methods of clonal propagation and reproductive biology of blue flowered species of *Conospermum*. Potential for commercialisation is high with test shipments receiving good response on overseas markets. Several species have small clusters of blue flowers in an attractive inflorescence and are a popular vase filler in Japan and the USA. Bush picking currently accounts for most of the annual harvest. The conservation status of blue flowered *Conospermum* varies, but populations are in decline and some species are under threat or becoming rare and endangered. Current methods of propagation have limited success. It is difficult to root these species *in vitro*, and strike rates from cuttings are low. The suitability was investigated of various explants of *Conospermum* for the induction of embryogenesis *in vitro*. In the field, plants grow for a short time as rosettes, then produce tall, leafless stems with small axillary buds that are initially vegetative and later convert to floral structure. *C. eatoniae* axillary buds excised from March to May sprouted to produce shoots. From June to early July bud explants produced shoots or callus, however when buds were excised in late July when they were floral, most died. Immature embryos collected in October also died in culture. In *C. caeruleum* buds from inflorescences with vegetative buds produced only shoots, and immature embryos died in culture. Zygotes from mature fruits of *C. caeruleum* showed direct embryogenesis on medium with BAP and TDZ.

P-1466

Effect of Different Phytohormones on Adventitious Shoot Regeneration in Transformed Root Cultures of *Centaurium erythrea* Gillib. ANGELINA R. SUBOTIC, Dragoljub Grubisic, and Snezana Budimir. Institute for Biological Research "Sinisa Stankovic", 29.novembra 142, 11000, Belgrade, Yugoslavia. E-mail: HEROINA@IBISS.BG.AC.YU

Centaurium erythrea Gillib., Centaury is a member of family *Gentanaceae*. This speciea is an object interest because of their pharmaceutical value, i.e., production of biologically active metabolites. Stable transformation and expression of transgenes was achived in Centaury using *Agrobacterium rhizogenes*—mediated system. Seedlings on cocultivation with *A. rhizogenes* strains A4M70GUS produced adventitious roots which showed rapid growth on hormone free-medium.Five hairy root clones exhibited the transformed phenotype. The extent of phenotypic change varied from clone to clone.The integration of Ri-plasmid T-DNA was confirmed by PCR analyses. Adventitious shoot regeneration was compared on media containing various auxins (IAA, IBA, NAA and 2,4-D), cytokinins (BA, CPPU, DPU, KIN, 2iP, ZEATIN and TDZ), ABA and GA_3. All phytohormones were compared at six concentrations (0.01, 0.03, 0.1, 0.3, 1.0, 3.0 microM).The type of cytokinins critically affected shoot regeneration, and CPPU, TDZ and KIN were most effective among the cytokinins tested. 2iP and DPU were least effective among all cytokininis tested and only limited number of explants responded. All tested auxins showed inhibitory effect on shoot formation. Light had a stimulatory effect on bud induction and development in the presence of both auxins and cytokinins.

P-1467

Somatic Embryogenesis and Plant Regeneration in Sago Palm (*Metroxylon sagu* Rottb.). J. S. TAHARDI and Imron Riyadi. Biotechnology Research Unit for Estate Crops, Bogor 16151, Indonesia. E-mail: jtahardi@indo.net.id

The sago palm (*Metroxylon sagu* Rottb.), a monocot tree native to equatorial swamplands, is fast becoming an important source of industrial starch. Sago palm can be grown from seeds but it is generally propagated by suckers. As sago palm cultivation is expanding on a plantation scale, it has become increasingly difficult to meet the demand for superior planting materials by conventional propagation. An alternative technology must be developed to enable production of elite clonal planting materials *en masse*. In this paper we report an efficient system for induction of somatic embryogenesis aimed at plant regeneration in sago palm. Shoot apical tissues from young suckers were cultured in a modified Murashige-Skoog (MMS) medium enriched by a combination of auxin and cytokinin, with 0.25% activated charcoal, 3% sucrose, and 2 g/L Gelrite. An auxin, picloram, was used at 5–160 mg/L in combination with 1 mg/L kinetin. After 1 month of culture in darkness, the explants expanded and began callusing. After another 2–3 months in the light, nodular structures appeared. The nodules were friable and highly proliferative upon subculturing. Transfer of these nodules to media with progressively reduced levels of picloram (1/5 and 1/10 of the original concentrations) led to the development of somatic embryos similar to those reported for oil palm. Germination of the somatic embryos and subsequent shoot regeneration were achieved in the same MMS medium but without phytohormones. Further research is underway to optimize and scale-up the procedure for efficient production of sago palm somatic embryos and propagules.

P-1468

Morphogenic Potential of *Cucumis melo* L. Cvr. Cantaloup. A. TIRADO PEREA and M. Perea Dallos. Departamento de Biología, Universidad Nacional de Colombia, P.O. Box 14490, Bogotá – Colombia. E- mail: andretirado@yahoo.com, mapere@ibun.unal.edu.co

The need to get better fruits for tropical regions and temperate zones, gives to the application of plant cells and tissue culture techniques.This research was carrying out studies to evaluate the morphogenic potential of *Cucumis melo* L. Cvr. Cantaloup using: shoot tips, cotyledons and nodal shoots. Plantlets obtained from seedlings by aseptic cultures of zygotic embryos, different explants were transferred to in vitro conditions using Murashige & Skoog (1962) – (M&S, 1962) with 30grs/L sucrose, agar-agar Merck (r) 8g/L and pH 5.8, all cultures were transferred at 27 °C ± 1 under 16/8 hours photoperiod.For shoot-tips we excised in aseptic conditions and transferred to (M&S, 1962) medium supplemented with 0 – 1.5mg/L of Indole-3-acetic acid; (IAA) and 30g/L of sucrose. Because of vitrification and callus growing it was necessary to use half strength of nitrogen. For better plantlets developed the IAA was eliminated.Shoot proliferation with nodal segments were induced in M&S with 6-Benzylaminopurine; (BAP) 0 – 1mg/L as cytokinine. We obtained six buds for each nodal segments.For direct morphogenesis the use of cotyledons of 1, 2, and 3 days age the use of Kinetin 1.5mg/L and sucrose 30g/L was very effective after 6 weeks with young cotyledons.The indirect somatic embryogenesis in the basal medium M&S supplemented with 2,4-dichlorophenoxyacetic acid; (2,4 – D) and 30g/L sucrose.The isolation of protoplasts was obtained from young cotyledons using Cellulase – Onozuka R- 10 w/v 2% after 15 minutes.

P-1469

Cryopreservation of Embryonic Cultures of *Picea mariana* (Black Spruce) Using A Vitrification Protocol. DARREN H. TOUCHELL, Vincent L. Chiang, and Chung-Jui Tsai. Plant Biotechnology Research Center, School of Forestry and Wood Products, Michigan Technological University, 1400 Townsend Drive, Houghton, MI 49931. Email: dhtouche@mtu.edu

This study reports on the first use of the vitrification procedure for the successful cryopreservation of embryonic cultures of a coniferous species. Embryogenic cultures of *Picea mariana* (Black spruce), had highest survival (50 to 67%) when callus clumps were precultured on sorbitol for 48 h followed by incubation in PVS2 cryoprotective vitrification solution at 0°C for 30 min and directly immersing in liquid nitrogen. Survival was also influenced by the concentration of sugars or sugar alcohols in the preculture media with 0.8 M sorbitol or 1.6 M glycerol being the most effective. No survival was obtained using sucrose or glucose. Further, using sorbitol and glycerol as preculture treatment, 9 out of 11 embryogenic lines successfully survived liquid nitrogen treatments.

P-1470

Advance Induction Somatic Embryogenesis in *Heliconia psittacorum*. C. VALENCIA and L. Atehortua. Instituto de Biología. Universidad de Antioquia. A.A.1226 Medellín (Colombia). Fax: (574)2638282. Email: cvalencia67@yahoo.com, latehor@quimbaya.udea.edu.co

Embryogenic callus of *Heliconia psittacorum*, ornamental tropical plant of high commercial value, was induced from *in vitro* plantlets buds using MS medium supplemented with 2–4 ml/L 2,4-D (dichloropheoxiacetic acid), 2–4 ml/L IAA (indolacetic acid) and 1–2 ml/L NAA (naphtaleneacetic acid) of the solution stock to 1000 mg/l under dark conditions. The embriogenic callus was maintained under continuous subculture in the same medium, but with the addition of 0.5–1 mg/L of calcium pantothenate. When the embriogenic callus was placed under laboratory artificial light conditions and free hormones medium, the culture developed globular callus followed by numerous coleoptilar embryos.

P-1471

Comparison of *Capsicum baccatum* and *C. annuum* for Stable Transformation Using *Agrobacterium rhizogenes*. LUIS VALERA and Gregory C. Phillips. Molecular Biology Department, New Mexico State University, NM 88003. E-mail: grphilli@nmsu.edu

The objective of the present work is to find an appropriate method for stable *Capsicum* transformation using *Agrobacterium rhizogenes*. In our lab, cultures of cotyledons, inverted hypocotyls, leaves, half seeds, organogenetic callus, and hypocotyl hooks of *C. baccatum* cv 'Campanita' exposed to *A. rhizogenes* strain K599 resulted in different degrees of stable transformation. The experiments done with *C. annuum* cv "NM6–4" included zygotic embryos but did not include organogenetic callus. All explants were precultured for 2 days on semisolid MS containing with BA (5 mg/L), IAA (1 mg/L), GA_3 (2 mg/L), and Citric and Ascorbic acids (100 mg/L each). This was followed by a 48 hour coculture period on the same medium before transferring the explants to selective medium (same as above) that included appropriate antibiotics. Measurements of regeneration response were done at 40 and 60 days after coculture, and after that the explants were assayed for GUS expression. The analysis of *C. baccatum* explants showed that 4% of inverted hypocotyls and 1.5% of hypocotyl hooks had transformed but ill-defined buds. On the other hand cotyledons, leaves, half seeds and organogenetic callus did not have a positive transformation response. In the case of *C. annuum* zygotic embryos showed 1.2% transformation on the hypocotyl region, but hypocotyl hooks showed 0% GUS expression. In general, the results of both *C. baccatum* and *C. annuum* are pointing to various forms of hypocotyl explants as the most appropriate explants under our transformation conditions. The poorly defined adventitious buds are a serious limitation for obtaining complete plants, but experiments are underway to attempt to solve the problem. Finally, similar experiments using *A. tumefaciens* will be completed soon for additional comparisons.

P-1472

High Efficiency Transformation Protocol for Three Indian Cotton Varieties via *Agrobacterium tumefaciens*. VENKATA SAVAYATHI VALLURI, V. Prasad, B. Gita Lakshmi, and G. Lakshmi Sita. Department of Microbiology and Cell Biology, Indian Institute of Science, Bangalore, India, 560012. E-mail: satya@mcbl.iisc.ernet.in

A protocol for consistent production of transgenic cotton plants in three Indian varieties was established utilizing Agrobacterium mediated transformation. Shoot tip explants were transformed by cocultivation with *Agrobacterium tumefaciens* strain LBA 4404. The strain harbours a binary vector pBAL2 carrying the reporter gene GUS intron (GUS-INT) and the marker gene neomycin phosphotransferase (NPTII). Regeneration potential of explants on different hormones was studied in detail. Among the different combinations of hormones tested, BAP and NAA in the medium influenced efficient regeneration of shoots by organogenesis. Shoot bud proliferation and elongation was achieved in 3–4 weeks time on medium supplemented with GA_3. The putatively transformed shoots were harvested and placed for rooting on medium containing IBA and 75mg/l kanamycin. Transgenic plants were recovered in 12–16 weeks from the time of gene transfer to establishment in pots. Molecular analysis of the field established plants was carried to confirm the transgenic nature. The presence of GUS and NPTII genes in the transgenic plants was verified by histochemical GUS assay and PCR analysis respectively. Integration of T-DNA into the genome of putative transgenics was further confirmed by Southern blot analysis. A total of 70–75 transgenic plants were raised in pots. Progeny analysis of these plants showed a classical Mendelian pattern of inheritance for NPTII gene and PCR analysis of R_1 plants confirmed the inheritance of the introduced gene.

P-1473

Effect of Ammonium Nitrate, Water Availability and Applied Cytokinins on Hyperhydricity of Micropropagated *Aloe polyphylla*. Mariana V. Ivanova, A.V. Ramarosandratana, and J. VAN STADEN. Research Centre for Plant Growth and Development, University of Natal—Pietermaritzburg, Private Bag X01, Scottsville 3209, South Africa. E-mail: vanstadenj@nu.ac.za

Aloe polyphylla is an endangered species as it isintensively used in traditional medicine and culture. Due to its high potential as an ornamental plant, in vitro propagation techniques were developed as part of a conservation program. Malformation due to hyperhydricity posed a problem with Aloe polyphylla. Some parameters of the culture medium: ammonium nitrate, water availability, gelling agents and cytokinins were investigated in order to evaluate their effect on hyperhydricity and multiplication rate. Raising the concentration of ammonium nitrate in the culture medium from 10 to 60mM increased the percentage of hyperhydric shoots from 4 to 22% but decreased the multiplication rate. Decreasing water availability by increasing the concentration of gellan gum from 0 to 1.6% (w/v) did not avoid hyperhydricity. The multiplication rate increased with more gellan gum. Media supplemented with BA had a lower percentage of hyperhydric shoots than with zeatin or TDZ. The multiplication rate was highest on media containing zeatin or BA. BA was the best cytokinin, yielding a high multiplication rate with low hyperhydricity. Media solidified with agar had fewer hyperhydric shoots (14%) and higher multiplication rates (17 shoots per explant) than media having the same water availability but gelled with gellan gum (45% and 7 shoots per explant respectively). As with other species, agar displays some protective properties against hyperhydricity in Aloe polyphylla.

P-1474

Tri-iodobenzoic Acid Promotes Embryonal Suspensor Mass (ESM) Initiation from Hypocotyl Segments of Norway Spruce Embryos. A. V. Ramarosandratana and J. VAN STADEN. Research Centre for Plant Growth and Development, University of Natal-Pietermartizburg, Private Bag X01, Scottsville 3201, South Africa. Email: vanstadenj@nu.ac.za

Mature zygotic embryos are used when studying somatic embryo initiation *Picea abies* as their response towards the induction treatments is high. Moreover, seeds stored in a controlled environment remain available longer while being protected from temperature and humidity fluctuations. Mature zygotic embryos were segmented into four parts: Z1 (cotyledons and shoot apical meristem), Z2 – Z3 (hypocotyl) and Z4 (root cap) along the apical-basal axis of the embryo. The orientation of these segments on the medium influenced the expression of their embryogenic capacity. Therefore, possible involvement of auxin transport during initiation of ESM was investigated. This was done by the testing the influence of the auxin transport inhibitor 2,3,5-triodobenzoic acid (TIBA) on ESM. The upper hypocotyl (Z2) was the most embryogenic zone yielding 20% initiation without TIBA. Low concentration of TIBA (2.5–5μM) increased the initiation rate from 20 to 45% for Z2 whereas concentrations of ≥ 40μM totally inhibited ESM formation. TIBA also promoted Z3 and Z1 when these explants were oriented in apical or basal-end-up positions. After two months of culture, callus proliferation was significantly higher on hypocotyl zones Z2 and Z3. Callus formation and proliferation decreased when the TIBA concentration was increased from 5 to 80μM. Thus, the response of hypocotyl segments of Norway spruce embryos to TIBA was biphasic in that lower concentrations stimulated ESM initiation, but higher concentrations inhibited ESM initiation by reducing callogenesis.

P-1475

Transformation and In Vitro Response of 38 Current European Winter Wheats (*Triticum aestivum* L.). A. VARSHNEY and F. Altpeter. Institut fuer Pflanzengenetik und Kulturpflanzenforschung Gatersleben, Corrensstr.3, D-06466 Gatersleben, Germany. E-mail: varshney@ipk-gatersleben.de

For crop improvement through biotechnological approaches, it is desirable to use a productive genetic background. So far, only some highly responsive model cultivars could be transformed which lag far behind from the elite germplasm in agronomic perfomance. In this work, 38 European winter wheat cultivars or breeding lines were examined for their suitability for biolistic transformation. The variation in the number of regenerated plants per immature embryo turned out to be highly significant ($p<0.01$) among the tested genotypes. Independent transgenic plants were regenerated from nine winter wheats with 0.2% to 2.0% tranformation frequency by use of the bar gene as selectable marker and bialaphos as selective agent. These nine winter wheat genotypes included a recently released high-yielding, disease-resistant cultivar (Certo), well-established cultivars with elite bread-making quality (cultivars Tarso, Alidos) and current breeding lines differing in yield, disease resistance and grain quality. The independent transgenic wheat lines could be confirmed by Southern blot analysis, polymerase chain reaction, phosphinothricin acetyl transferase activity assay and herbicide application. In the majority of lines, transgene expression showed mendelian segregation. The lines which were found to be suitable in the scope of this study will also be useful in future crop improvement programs.

P-1476

Somaclonal Variation in *Arabidopsis thaliana*. A. M. VAZQUEZ, J. Rueda, G. Muñoz, E. Ruiz, A. Peñalosa, R. Linacero, and F. J. Espino. Departamento de Genética, Facultad de Biología, Universidad Complutense, 28040-Madrid, Spain. E-mail: anavaz@bio.ucm.es

Arabidopsis thaliana regenerated plants were obtained from root cultures of ecotypes Columbia (Col) and Landsberg erecta (Ler). Four different approaches were followed in order to assess which one is the most accurate in the detection of the occurrence of somaclonal variation among the regenerated plants, namely the analysis of the chromosome number, the study of RAPD patterns, the study of the PCR amplification patterns generated with F13 and F17 primers (Diaz-Perales et al, 2001, BioTechniques 30, 718–720) and the PCR-RFLP analysis of two hypervariable regions of the chloroplast genome. Neither the RAPD patterns nor the PCR-RFLP analysis showed any differences within the Col or the Ler regenerated plants. Regarding the chromosome number, 40% of the Col regenerated plants and 64% of the Ler regenerated plants showed a variable number of tetraploid cells in the leaves, ranging from 0.2 to 9% in Col and from 0.2 to 4.8% in Ler. The amplification pattern obtained when F17 was used as primer showed an extra band of 490 bp in 1.9% of the Col regenerated plants and an extra band of 390 bp in 12% of the Ler regenerated plants. When F13 was used as primer, no differences were observed among the plants within each ecotype. These results indicate that PCR with the primer F17 is the most suitable approach for the purpose of early detection of somaclonal variation.

P-1477

Clonal Propagation of Hybrid Sweetgum (*L. styraciflua* X *L. formosana*). W. A. VENDRAME*, C. P. Holliday**, S. A. Merkle**. *Tropical Research and Education Center, University of Florida, Homestead, FL 33031 and **D. B. Warnell School of Forest Resources, University of Georgia, Athens, GA 30602.

Somatic embryogenic cultures of hybrid sweetgum (*L. styraciflua* X *L. formosana*) were initiated from immature zygotic embryos on induction culture medium. Prolific embryogenic cultures where obtained in which repetitive somatic embryos were formed and regenerated into plantlets. Plantlets were evaluated and confirmed for hybrid genotype by RAPD analysis and leaf morphology evaluation. Stomatal analysis was performed revealing differences in stomata size and stomata number per leaf area for hybrid plantlets and parental species. A protocol for cryopreservation of hybrid embryogenic cultures was efficiently implemented, providing rates of regrowth near 100% for all samples. Cryopreservation will allow the long-term storage of viable embryogenic cultures, while hybrid trees are field tested for performance and identification of superior genotypes. Overall, 308 hybrid culture lines were initiated, representing 21 crosses between *L. styraciflua* and *L. formosana*, with a regeneration rate of 7.1%. However, 67% of all embryogenic cultures were converted into plantlets. In this work we report somatic embryogenesis as a feasible approach for clonal propagation of hybrid sweetgum.

P-1478

The Effect of PPM (Plant Preservative Mixture) on *Agrobacterium*-mediated Maize Transformation. AMANDA K. VINAS, Jeffrey R. Lane, Cory D. Davis, Michael E. Horn, Elizabeth E. Hood. ProdiGene, 101 Gateway, Suite 100, College Station, TX 77845. E-mail: mherbert@prodigene.com

The production of recombinant proteins through the use of transgenic plants is increasing as a viable pursuit in the pharmaceutical and industrial enzyme arenas. With the popularity of this technology growing, it is imperative that companies and research organizations gain a full understanding of the contamination control options available for their transformation systems. Various antibiotics have been tested for use in the *Agrobacterium*-mediated maize transformation system to control A. tumefaciens overgrowth and fungal contaminants on sucrose -rich media. PPM (Plant Preservative Mixture) was examined as a control for both of these problems. PPM contains two isothiazolones (methylchloroisothiazolinone and methlisothiazolinone) and is classified as a biocide. PPM at some levels has been reported to have phytotoxic properties on some plant tissues and stages of plant growth. Eight levels of PPM (0–7.5 mL/L) were tested for their effects on various stages of maize development, and on frequent lab contaminants. The PPM was highly effective in controlling fungus and other lab contaminants including *Agrobacterium* at levels as low as 1.5mL/L. It was not found to be phytotoxic at levels as high as 5mL/L on immature zygotic embryos, and transgenic callus. However, at the 7.5mL/L level, callus growth, embryo root formation, and embryo callusing appeared to be stunted. Current experiments are in progress to test the efficacy of PPM in a transgenic maize production system.

P-1479

Pollen Related Food Allergy: Regeneration of Sweet Cherry (*Prunus avium* L.) as a First Step to Establish an In Vitro Model to Reduce Allergenicity by Antisense RNA. A. MATT, A. Reuter*, S. Vieths*, and J. A. Jehle. Biotechnological Crop Protection, SLFA Neustadt/Weinstraße and *Department of Allergology, Paul-Ehrlich-Institut, Langen. E-mail: amatt.slfa-nw@agrarinfo.rlp.de

The majority of hypersensitivity reactions against fruits and vegetables is highly associated with pollen allergies, in particular with birch pollen allergy. Approximately 8% of the population in Central and Northern Europe is sensitized to birch whereof 70% suffer from food allergies. Sweet cherry was selected as a model to develop a plant with reduced allergenicity because of structural similarity of the main cherry allergen Pru av 1 with homologous allergens in birch and foods such as apple, pear and hazelnut as well as celery and carrot. Thus, the reduction of expression level of Pru av 1 concerns a widely spread allergen family which is relevant for the majority of pollen related food allergies in Europe. With the use of plant tissue culture we are able to proof if it is possible to use *in vitro* cultures as a model and than to obtain plants with reduced allergenicity in a short time. We examined cherry fruit protein extracts of eleven different cherry cultivars for their protein patterns as well as Immunoglobulin E (IgE) reactivity and expression level of Pru av 1. No significant differences were detected among all *in vivo* cultivars, using sera from cherry-allergic patients, applied in EAST-inhibition (enzyme-allergo-sorbent-test) and immuno-blotting. On account of this we selected five cultivars at random for starting *in vitro* culture. As an explant for establishing *in vitro* meristem tip cultures winter buds were prepared and put on five different media. The optimal plant growth and proliferation of axillary shoots was achieved with 1 mg/l BAP added to QL-medium. For regeneration experiments leaf and stem segments of all cherry cultivars were cultured on 1/2 DKW and 1/2 WPM, QL and MS media. Different combination of 2,4 D, BAP, TDZ, IAA, NAA and IBA were added to the above mentioned media for shoot induction. Plant regeneration from leaf segments of all cultivars was observed on eleven of 30 tested media whereas on 23 of 30 different media new shoots from stem segments of all cultivars were seen. Using leaves as a source for shoot induction 2% to 8% of the new shoots were induced according to the cultivar and the media. The regeneration efficiency using stem segments ranged from 4% to 66% depending on the medium and cultivar. Subsequent examination of protein extracts prepared from the different regenerated *in vitro* meristem tip cultivars indicated the presence of IgE-reactive Pru av 1 in leaves of regenerated plants. This finding indicates the suitability of applying an *in vitro* model for the development of hypoallergenic cherries using an antisense-RNA strategy, and to assess potential reduction of allergenicity at a very early stage of plant regeneration.

P-1480

In Vitro Propagation of Western Australian Seagrasses. J. G. WILSON and I. J. Bennett. Centre for Ecosystem Management, Edith Cowan University, Western Australia, 6027. E-mail: juliagwilson@hotmail.com

Seagrasses play a significant role in marine ecosystems by providing productive nursery areas for fish and juvenile crustaceans and the primary food source for dugong and green turtle. Their local importance includes their ability to stabilize sediments, provide detritus, filter water and provide a substratum for sessile biota. Despite this, little is known about their growth requirements and even less about propagation techniques. Three species of Australian seagrasses, *Halophila ovalis, Posidonia australis* and *P. coriacea*, have been initiated into tissue culture through sterilization of seeds. Cultures of *H. ovalis* have been maintained for over five years on a medium containing 1/2 MS basal salts, 60 mM sucrose, 20 $g.l^{-1}$ SIGMA seasalts, 10 mM 3-(N-Morpholino) propane-sulfonic acid, and 5 mM kinetin at pH 7.0. *P. australis* and *P. coriacea* have only been maintained on this medium for 12 months. Parameters tested for these species include seawater medium, salinity, substrates, carbon source, nitrogen source, hormones, pH, buffers, subculture length, temperature and light. The most important parameter found to improve growth and chlorophyll content of *H. ovalis* is maintenance of pH through the use of buffers. Buffers with a propane sulfate base produced optimal growth. For *P. australis,* seasalts at 30 $g.l^{-1}$ increased growth, as did the absence of a solid substrate. *P. coriacea* exhibited significantly more growth in seasalts than natural seawater and at 20, 30 and 40 $g.l^{-1}$ seasalts than 0 and 10 $g.l^{-1}$. The protocol developed is unusual with regard to micropropagation systems as whole plants are maintained with leaves, rhizomes and roots throughout the culture period. In addition, due to the aquatic nature of these plants, it is possible to measure oxygen evolution as an indicator of photosynthesis. This provides a unique opportunity to readily measure the parameters that are important for the development of autotrophic growth (e.g., carbon source, nutrients and light intensity). This will lead to a better understanding of the growth requirements of these plants and increase the success of transfer to field conditions.

P-1481

Genetic Transformation of *Cyclamen persicum* Mill.—Effects of Different Antibiotics on Callus Growth and Differentiation of Somatic Embryos. TRAUD WINKELMANN and Margrethe Serek. Institute of Floriculture, Tree Nursery Science and Plant Breeding, Herrenhaeuser Str. 2, D-30419 Hannover, Germany. E-mail: winkelmann@zier.uni-hannover.de

A reliable transformation system for *Cyclamen persicum*, an important ornamental pot plant in Europe and Japan, can be used to improve this species with regard to resistances to thrips and fusarium wilt e.g., but also flower colour or scent. To eliminate agrobacteria after the coculture cefotaxime and carbenicillin are the prevalently applied antibiotics. In cyclamen, 500 mg/l carbenicillin had a slightly negative effect on cell growth on solid medium, but a strong effect (80% reduction) in liquid media. Regeneration of somatic embryos also was affected by carbenicillin. On the other hand, the addition of 500 mg/l cefotaxime had no effect on cell growth and increased the number of developing somatic embryos on differentiation medium slightly. One essential step within a transformation protocol is the selection of transgenic cells. Resistance to kanamycin, conferred by the npt II (neomycin phosphotransferase II) gene, is commonly used to identify transgenic cells. In cyclamen at 150 mg/l kanamycin callus growth and differentiation were observed on solid medium, while in liquid culture 100 mg/l kanamycin reduced the growth of embryogenic suspension cultures to about on third, and after plating no somatic embryos differentiated from these. From this it has to be concluded, that selection with kanamycin at this level in solid media is insufficient and will lead to a high number of escapes while in liquid culture a selection using kanamycin is more efficient. In the differentiation medium kanamycin (100 mg/l) reduced the number of developing embryos per gram callus to 124 as compared to 322 in the control. If cefotaxime (500 mg/l) was added to the kanamycin containing medium, a mean number of 183 somatic embryos per gram callus regenerated, indicating that cefotaxime affected the selection efficiency of kanamycin.

P-1482

Micropropagation of *Exochorda racemosa*. GUOCHEN YANG and Marihelen Kamp-Glass. Department of Natural Resources & Environmental Design, North Carolina A&T State University, Greensboro, NC 27411. E-mail: yangg@ncat.edu

The objective of this research was to enhance axillary shoot proliferation of pearlbush, an ornamental shrub. Softwood explant materials were used to initiate the *in vitro* cultures. Woody plant medium (WPM) plus BA at 0.1 mg/l, 3% sucrose and 0.7% agar with the pH of 5.8 was used as the basic medium to initiate and maintain the cultures for further studies of plant growth regulators for shoot multiplication. The micropropagated shoots were then cut and transferred to media containing BA, CPPU or thidiazuron (TDZ) at different concentrations. Those cultures from the media containing TDZ appear to produce the best shoot proliferation. Statistical analysis will be made to determine the best growth regulator(s) tested with optimum concentrations for *in vitro* shoot production. All cultures were transferred onto fresh media every 4 weeks and maintained under a 16-hour photoperiod and a photo flux density of 12.8 $\pm$ 4.8 Mol $s^{-1}m^{-2}$ light provided by cool white fluorescent tube at 23 $\pm$ 3°C.

P-1483

Cassava Shoot Growth and Root Development on Basal Nutrient Media. T. W. ZIMMERMAN, Latashia Joseph, Jacqueline A. Kowalski. University of the Virgin Islands Agricultural Experiment Station, RR 2 Box 10,000, Kingshill, VI 00850. E-mail: tzimmer@uvi.edu

Cassava (*Manihot esculenta*) is normally field grown by planting leafless 30 cm stem segments into the soil. This study was conducted to evaluate the effect of basal salts, without plant growth regulators, on shoot growth and root development from nodal segments of cassava cultivar TMS 60444. The basal media were Murashige and Skoog (MS), 1/2 MS, Driver and Kuniyuki (DKW), Quoirin and Lepoivre (QL) and Loyd and McCown (WPM) each with 30 g/L sucrose and 2 g/L Phytagel and adjusted to pH 5.8. Nodal segment from in vitro grown plants were used as explants. The MS and 1/2 MS outperformed the other three media formulations. Within three weeks, both the MS and 1/2 MS had produced an average of 4 roots, >1cm, and two fully expanded leaves. One fully expanded leaf was produced on QL after 4 weeks. However, root growth was suppressed in DKW, QL and WPM. Using nodal explants on MS or 1/2 MS, rooted shoots for transfer to pots can be produced within four weeks.

P-1484

The Agricultural Biotechnology Support Project (ABSP): Building Capacity in Biotechnology Research and Policy. JOHAN A. BRINK, Andrea Johanson, and Karim Maredia. Institute of International Agriculture, 319 Agriculture Hall, Michigan State University, East Lansing, MI 48824. E-mail: brinkj@msu.edu

The Agricultural Biotechnology Support Project (ABSP) is a USAID-funded project based in the Institute of International Agriculture at Michigan State University (MSU). Since 1991, ABSP has integrated research and policy development to assist developing countries in biotechnology research and in establishing regulatory frameworks for biotechnology in agriculture. This was implemented through MSU, in collaboration with other U.S. universities, the private sector and developing country institutions. The ABSP applied research projects have included: geminivirus resistance in cucurbits for Egypt; virus resistance in sweet potato for Africa; potato tuber moth resistance in potatoes; and Asian corn borer resistance in tropical maize. The ABSP has provided technical assistance and capacity building in the development of policy frameworks that support the practical application of biotechnology. The direct linkage between crop biotechnology research and policy through the ABSP has been important in providing an incentive to move national policy development forward. The ABSP has initiated training and capacity building activities directed toward the establishment of institutional and national legal systems for intellectual property rights protection in agriculture, has provided assistance at the institutional level to improve the ability of public research institutions to negotiate agreements with the local and international private sector and has managed IPR issues associated with technology access. The ABSP has also provided technical assistance at the national and institutional levels to develop and implement biosafety regulations. This has facilitated the field-testing of genetically engineered crops in several countries. The development of regional biosafety capacity building programs in Eastern and Central Africa and in the Southern Africa region is currently being supported by the ABSP.

P-1485

Cotton Biotechnology and Genetic Engineering in China. BAOHANG ZHANG. University of Texas at Austin, Austin, TX 78703. Email: zbh68@mail.texas.edu

During the past two decades, Chinese scientists have made great progress in cotton biotechnology and genetic engineering. They obtained firstly regenerative plants from cotton anther and protoplast culture in the world, and also obtained regenerated plants from many domestic elite cotton varieties. After transgenic cotton carrying the insect-resistant (Bacillus thuringiensis: B.t.) gene was commercialized in 1996, at least ten Bt-cotton varieties were planted in China. In 2000, there were over 1,000,000 hectares of Bt-cotton were planted. Two kinds of bivalent insect-resistant transgenic cotton have been obtained. This new bivalent insect-resistant transgenic cotton carried two insecticidal genes, B.t. gene and CpTI gene, or pea lectin (P-Lec) gene and soybean Kunitz trypsin inhibitor (SKTI) gene respectively, and was commercialized in 2000. Herbicide-resistant varieties for 2,4-D and Bromoxynil are under development and are expected to reach the market by 2002. Disease-resistant transgenic cotton is under development and testing in lab and fields, and is reached the market by 2000. Fiber improvements, stress resistance, and male sterility and fertility for hybrid cotton are the next targets for cotton biotechnology. Several genes for fiber improvement and hybrid cotton are being tested in various laboratories. New genes for insect, herbicide and disease resistance are being sought.

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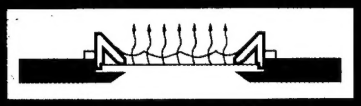
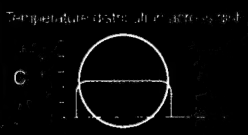
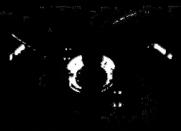
The Delta T Culture Dish utilizes an optically transparent electrically conductive coating on the underside surface of the coverslip-bottomed dish that efficiently transfers heat by conductivity to the cells.



Coverslip bottomed, self-heating glass surface, low dead-volume dish





Think of it as a precision, miniature, coverglass bottomed hot tub for your cells!

Delta T4 Dish Accessories

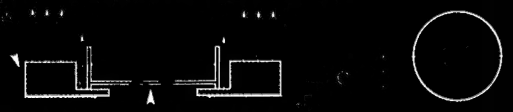
Gas segregation localization made easy! Optically Flat Glass Culture Cylinders Hydrostatically Seal Without Grease!

Heated Lid minimizes condensation and includes CO2 Port

Coverglass lid forms optical surface above cells for uniform contrast-free optical images. Standard Lid (top) and perfusable version (bottom) available

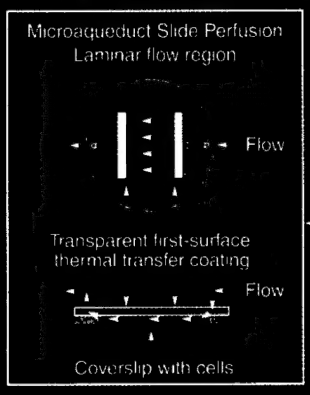
Obsolete Culture Dish Heating

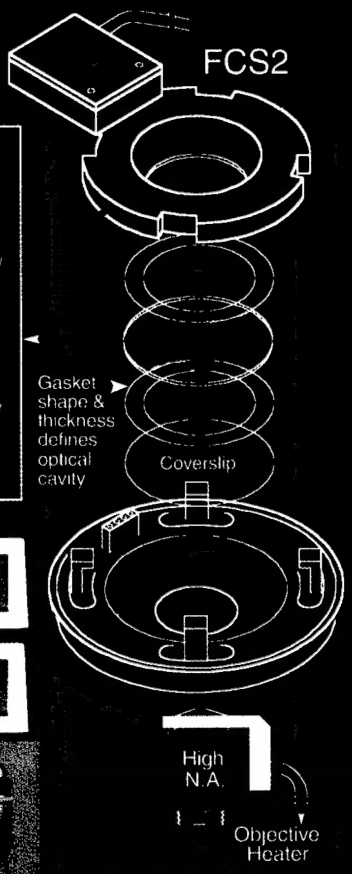
Non-uniform heat distribution, not high N.A. compatible, slow temperature transfer, unstable in Z axis and subject to surface evaporation induced temperature changes

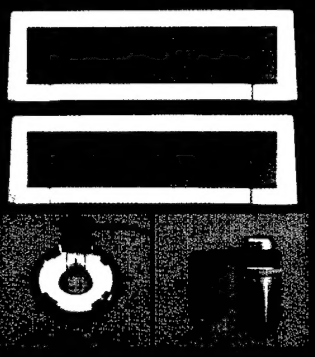


Traditional ordinary plastic culture dish

FCS2 Closed System Micro-Observation Environment







The FCS2 Closed System Chamber consists of a two-channel controller which regulates the temperature of the microaqueduct slide and chamber. (50) 40mm Coverslips (5) Microaqueduct Slides and (30) Gaskets. A separate controller for the objective heater is needed for use with high N.A. objectives.

Features include:

- Uniform temperature transfer direct to cells
- Temperature stabilization or recovery in seconds
- Compatible with all modes of light microscopy
- Laminar flow, perfusion with controllable cell surface shear
- Complete volume exchange as short as 1.0 second
- User definable volume & flow path
- No need for an air-curtain

Bioptechs now manufactures plain glass Delta TPG Culture Dishes. They are identical to standard Delta T Dishes without temperature control

3560 Beck Road, Butler, PA 16002 Web: http://www.bioptechs.com

Toll-Free 877-LIVE CELL Voice 724 282-7145 Fax 724 282-0745 Email: info@bioptechs.com